PROCEEDINGS

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Contents

S1 A National Forensic Sciences Enterprise and Transparency in Forensic Science: Legal and Practitioner Views on our Path Forward

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Educational Objective: The goal of this session is to take part in hearing the points of view from several diverse perspectives.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by developing awareness and hopefully consensus on the roadmap for our forensic science future.

An effective justice system is the cornerstone of freedom in the United States and abroad. Forensic science plays a critical role for which there is shared responsibility among the stakeholders in the criminal justice arena. The National Academy of Sciences recognized this important role and has articulated the critical need for strengthening forensic science in the United States. Efforts have been underway in the four years since the NAS Report to create our path forward to further strengthen forensic science. This is an opportunity to provide input and to help create our forensic science path forward.

Presenters from the Subcommittee on Forensic Science, along with representatives from the crime laboratory, judiciary, defense, and prosecution communities, will thoughtfully consider, discuss, and refine the future opportunities and challenges for forensic science. With a critical eye toward implementation, the infrastructure to support enhancing quality in forensic science, including standards and competency, will be paramount. The goal for this session is embodied in President Robert Barsley's theme for the Annual Meeting "*The Forensic Sciences: Founded on Observation and Experience, Improved by Education and Research,*" as well as the objectives of the American Academy of Forensic Sciences: *To promote professionalism, integrity, competency, education, foster research, improve practice, and encourage collaboration in the forensic sciences.*

Subcommittee on Forensic Science — Kenneth Melson, Professorial Lecturer in Law at the George Washington University Law School will discuss the forensic science issues studied by the NSTC Subcommittee on Forensic Science. These issues provide a foundation to meet the needs of the criminal justice system through a uniform, national forensic science enterprise. Kenneth Melson comes to us with a background in law and forensic science as a state and federal prosecutor, Past President of the AAFS, a former and long-time member of the ASCLD/LAB board of directors. He also served as the co-chair of the Subcommittee. Robin Jones will discuss the need for robust standards development processes and their use by Scientific Working Groups. Robin Jones is the principal of RWJ Consulting Services and has served as the Executive Secretary for the Subcommittee on Forensic Science, the Executive Assistant for the National Commission on the Future of DNA Evidence established by Attorney General Reno, and developed and executed Attorney General Ashcroft's Initiative on DNA Laboratory Backlogs. She has also contributed to the development of many publications and training tools for the National Institute of Justice in the area of forensic science.

Crime Laboratory Perspective — Consensus and cooperation from the entire range of forensic science service providers, from full-service crime laboratories to law enforcement identity units, are essential to advancing the quality of forensic science. Dean Gialamas is the Director of the Los Angeles County Sheriff's Department Scientific Services Bureau and has over twenty-two years of experience in forensic science. He is an accomplished author and instructor, and has been a leading-edge innovator in forensic science laboratory management. His insights will reveal the impact that forensic science reform will have on the quality and efficiency of the crime laboratory's work and whether the proposed reforms have gone too far, or not far enough.

Prosecution and Defense Perspectives — Advocates representing the defense and the state have important perspectives helpful to the creation of a fair and balanced system of adjudication. Steve Benjamin, President of the National Association of Criminal Defense Lawyers and a Richmond, VA, defense lawyer will explicate the needs of the defense community and the impact of the recommendations for improving forensic science. As Special Counsel to the Virginia Senate Courts of Justice Committee, a member of the Virginia Board of Forensic Science, the Virginia Indigent Defense Commission, and a Past President of the Virginia Association of Criminal Defense Lawyers, Mr. Benjamin is a 2003 recipient of the Virginia State Bar's Lewis F. Powell Pro Bono Award. He was also counsel in the landmark Virginia Supreme Court decision recognizing a constitutional right to forensic expert assistance at state expense for indigent defendants.

Michael Ambrosino is Special Counsel for DNA and Forensic Litigation in the United States Attorney's Office for the District of Columbia. Since 1989, he has been a federal trial attorney and prosecutor in the civil, fraud, homicide, and appellate divisions of the office. In 2010, Mr. Ambrosino was designated by the United States Attorney as Special Counsel to represent the United States in cases involving DNA and other forensic science disciplines. His duties include handling *Frye* and *Daubert* hearings, training assistant prosecutors, coordinating their work with crime laboratories, and overseeing post-conviction DNA testing. His perspective will reflect the challenges prosecutors have in using forensic science in investigations and in court proceedings as well as the ways laboratories can help ensure admissibility of their evidence and just verdicts.

Judicial Perspective — Judge Barbara Parker Hervey was elected to the Texas Court of Criminal Appeals in November 2000. Prior to her election, she was Assistant Criminal District Attorney in the Bexar County District Attorney's Office, Appellate Section for 16 years. In 2008, Judge Hervey formed the Texas Criminal Justice Integrity Unit to review the strengths and weaknesses of the Texas criminal justice system. She served on the Governor's Ad Hoc Committee to Rewrite the Texas Code of Criminal Procedure and received the Appellate Advocacy Award, Regional Awards, Region VI, Association of Government Attorneys in Capital Litigation. She was honored with a Certificate of Appreciation from the San Antonio Police Officers Association, for work on Jonathan Moore vs. State of Texas (Capital Murder of SAPD Officer Fabian Dominguez).

Judge Reggie Walton, United States District Judge for the District of Columbia, was appointed to Judge of the United States Foreign Intelligence Surveillance Court as well as served as the Chairperson of the National Prison Rape Reduction Commission and on the federal judiciary's Criminal Law Committee. While serving on the Superior Court, Judge Walton was the court's Presiding Judge of the Family Division, Presiding Judge of the Domestic Violence Unit and Deputy Presiding Judge of the Criminal Division. Judge Walton also served as President George H.W. Bush's Associate Director of the Office of National Drug Control Policy and as President Bush's Senior White House Advisor for Crime.

Forensic Science, NAS Report, Forensic Standards

S2 Applications of Education and Research to the Diverse Field of Forensic Science

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Educational Objectives: The goals of this special session are to provide forensic science students and professionals with five years or fewer experience an opportunity to learn about the various applications of education and research in forensic science at a day-long session, to present their own work/research at a Bring Your Own Slides session and in a poster session, to learn the skills required to be a successful expert witness at a Breakfast session, and to have an opportunity to network with fellow students, professionals, and AAFS members.

Impact on the Forensic Science Community: This session will impact the forensic science community by demonstrating the vast career paths available in forensic science, how education and research apply to the field, and by providing young professionals with the tools needed to successfully contribute to the forensic science field.

For more than a decade, the Young Forensic Scientist Forum (YFSF) has provided a program for a group of students in both undergraduate and graduate programs, and forensic scientists with less than five years of professional experience. The goal of YFSF and the Special Session is to provide the participants the tools required to be a success in the field of forensic science. The session allows participants to interact with their peers as well as the professional speakers and to build professional relationships that can span a career. Special Session topics provide attendees with a broad outlook at the many opportunities in the field of forensic science. In addition to the special session, YFSF offers two opportunities for young forensic scientists to present their own work or research, the first being the YFSF Bring Your Own Slides (BYOS) session and the second, the YFSF Bring Your Own Posters (BYOP) session. In addition, the Forensic Sciences Foundation's Emerging Forensic Scientist Award Winner is always invited to present their award winning paper.

For the AAFS 65th Anniversary Meeting in Washington, DC, the YFSF Special Session will present: "Applications of Education and Research to the Diverse Field of Forensic Science." The special session will be held on Tuesday, February 19, and include speakers who will discuss the various applications of education and research and the vast career paths within the field of forensic science by presenting their unique casework experiences and career choices. Through the presentations, speakers will demonstrate the different paths a forensic student or scientist can choose to take. The session will include speakers from many of the AAFS sections, highlighting the many opportunities the field may offer. Lunch is provided to both attendees and speakers who are registered for the special session.

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 conducted in a poster format.

The annual YFSF BYOS session takes place the evening of Wednesday, February 20, and will include presentations from students and new forensic scientists. YFSF does not require presenters of YFSF BYOS and BYOP 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 Thursday, February 21, with the annual YFSF breakfast session which includes a resume review panel. The breakfast session, which traditionally focuses on professional development, will continue with that theme by presenting "The Role of a Forensic Scientist as an Expert Witness" and will focus on the requirements of expert testimony as a forensic scientist and what it means to be an expert. Following the speaker presentations, professionals throughout the Academy will be available to review resumes, answer questions, and give advice on how to best highlight education and career achievements. As in the past, the Special Session allows students, young professionals, and AAFS members a comfortable setting to foster a career-long relationship throughout the weeklong events. Participants will be encouraged to apply for membership to AAFS and will be given guidance on the many opportunities available to aid in career enrichment.

YFSF, Special Session, Diverse

ES1 150 Years — Does Time Bring Agreement? The H.L. Hunley, the R.M.S. Titanic, and the Assassination of John F. Kennedy

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Educational Objective: After attending this presentation, attendees will: (1) have a greater understanding of the facts surrounding these events; (2) better understand the uncertainty surrounding them; and, (3) be able to draw conclusions about these events and the uncertainty surrounding them.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by highlighting known and unknown facts and areas for discovery in these three events—headline news in their day—still retaining great interest today.

The loss of the H.L. Hunley, the assassination of President John F. Kennedy, and the loss of the R.M.S. Titanic were newsworthy and were investigated to the extent possible at the time of their occurrence. However, even modern science cannot answer all of the questions raised. This presentation will highlight new discoveries and continuing questions.

H.L. Hunley: In 1864, a Confederate crew boarded the Hunley near Charleston, SC, to launch an attack on the Federal fleet blockading the harbor. A spar-mounted mine filled with black powder was targeted for the USS Housatonic stationed four miles off-shore. Even though spotted by sentries, the surprise submarine attack was so swift that the seamen onboard the Housatonic had no time to train the ship's guns on the Hunley. Handheld weapons fired a barrage of bullets that could not stop the attack. The submariners detonated the mine beneath the ship which sank in moments, becoming the first known submariners to successfully destroy an enemy ship in battle. After the attack, the submarine and its crew mysteriously vanished until 1995, when the Hunley was found buried on the seabed near the wrecked Housatonic. In 2000, the submarine was recovered and brought to the Warren Lasch Conservation Center in North Charleston where an interdisciplinary team performed the excavation, study, and conservation of the Hunley and the remains of its eight-man crew. A primary question the team hoped to answer was what happened to the crew and the submarine on that fateful night. Multidisciplinary collaborations have resulted in the development of a series of new methods and techniques for forensic site reconstruction, documentation, and interpretation which may be useful in modern crime scene investigations.

R.M.S. Titanic: The loss of the "nearly unsinkable" R.M.S. Titanic probably was considered the first mass fatality incident in modern times. Could the chain of events that led to the catastrophe and massive loss of life have been avoided? In the aftermath of the disaster, beginning with the discovery of the sunken ship by Robert Ballard in 1985, much has been explored and documented. Fifteen years of research and numerous expeditions to the wreck site detail how the ship was damaged and sank. Laboratory analysis, computer modeling, and a review of historical documents provide the evidence leading to a conclusion that a combination of management decisions, engineering design, sub-standard materials, combined with a one-in-a-million encounter with an iceberg led to this famous maritime disaster. Details on artifact recovery, conservation, and exhibition will be presented.

John F. Kennedy: It is astonishing that the interest and fascination with the assassination of President John F. Kennedy almost 50 years ago remains so intense and widespread. Few events have generated as much independent research, enthusiastic discussion, and passionate controversy. The event—originally witnessed by several hundred people, and over the past five decades, viewed by tens of millions (via the Zapruder film)—has remained the subject of doubt and outright rejection of the conclusions and official explanation of the responsible investigative governmental entity, the Warren Commission Report (WCR). Polls reveal 70-80% of the American public disbelieve the report. What reasons exist for this negative response? Numerous mistakes were made during the autopsy, performed by a pathologist not trained or certified to conduct autopsies on gunshot victims. In 1977, the U.S. House Select Committee on Assassinations appointed a nine-member autopsy panel of forensic pathologists—all Fellows of the American Academy of Forensic Sciences—to independently re-evaluate the autopsy findings. It concluded that two, and only two, bullets struck the President from behind and that their trajectories showed that they were fired from the Texas Book Depository Building where three discharged casings were found. Yet, many reject the "single bullet theory" that one bullet produced seven wounds in President Kennedy and Governor John Connally. Should this investigation be re-opened?

Titanic, Hunley, John F Kennedy

BS1 Grant Funding Opportunities in the Forensic Sciences for Academic Programs

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Educational Objectives: After attending this presentation, attendees will be able to: (1) identify grant funding providers; (2) locate grant funding opportunities on the Internet; and, (3) describe the current and planned activities of the Council of Forensic Science Educators (COFSE).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing awareness of grant funding opportunities in forensic science for academic programs.

Most public crime laboratories primarily focus on performance of casework and providing testimony in support of that mission. Laboratories typically also have limited resources for basic research and continuing professional education. Many suffer from lack of instrumental resources that can be dedicated to research and lack of personnel to dedicate to method development, evaluation, validation of new technologies, and technology transfer.

Partnerships between academic programs in forensic science and laboratories performing casework offer opportunities that benefit both parties, and these relationships are encouraged by the Forensic Science Education Programs Accreditation Commission (FEPAC) and the National Academies of Science (NAS) report from 2009. Forensic science as a field is highly applied and while forensic science practitioners rely on basic research in support of their methods and opinions, very little of that primary research is performed in public crime laboratories. Academic programs have well-established infrastructure to facilitate funded research and to assist with the transfer of technology from the research environment to its application in casework.

The Council of Forensic Science Educators (COFSE) is a group of program directors and faculty from forensic science programs that promotes accreditation and strong academic standards in forensic science programs. The group seeks to share resources and best practices to improve the quality of forensic science research and education. It also aims to help members identify funding opportunities and partnerships to support their programs. COFSE meets during the annual AAFS meeting and all forensic science educators are encouraged to attend.

COFSE has invited representatives of the National Institute of Justice (NIJ), the National Institute for Standards and Technology (NIST), and the Forensic Science Center of Excellence at Research Triangle Institute (RTI) to describe their granting philosophy, programs, and grants that may offer opportunities for academic programs.

Grants specifically for the forensic sciences are available through the NIJ, NIST, and RTI, among other groups. In 2011, more than $200 million were allocated to the forensic sciences for research in forensic labs and educational institutions, travel for training, training of law enforcement personnel, laboratory improvements, backlog projects, instrumentation, and other needs. There is funding available to students and educators that some may not know about and is within reach. This presentation will include speakers from various organizations that award grants to the forensic science community. With better awareness of opportunities that exist for funding, academic programs have the opportunity to contribute to the overall improvement and development of forensic science.

Grants, Education, Forensic Science

BS2 Quincy vs. Ducky II: The Rematch — An American Forensic Pathologist and a British Home Office Pathologist Face Off Again

Stuart J. Hamilton, FRCPath, East Midlands Forensic Pathology Unit, Level 3 Robert Kilpatrick Bldg, Leicester Royal Infirmary, Leicester, UNITED KINGDOM; and Wendy M. Gunther, MD*, OCME, Tidewater Dist, 830 Southampton Ave, Ste 100, Norfolk, VA 23510-1046*

Educational Objectives: The goal of this presentation is to provide an entertaining breakfast session of verbal sparring between two pathologists. More importantly, this presentation will provide an insight into the differences between how expert evidence (and indeed experts themselves) are treated in the United States and Great Britain, as well as providing the relative requirements in the two jurisdictions to be considered an expert and the different ways that expert witnesses interact with legal representatives of the different parties in the case.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by showing how, despite the substantial differences between the two systems, the education, experience, and up-to-date knowledge of relevant research of the pathologists can be applied to the criminal justice system in a manner that serves the ultimate goal of each system, the appropriate administration of justice.

In Chicago in 2011, the might of the United States and the majesty of the United Kingdom went toe-to-toe in a contest to decide once and for all which country had the best medicolegal system. On the side of the Stars and Stripes was Dr. Wendy Gunther, the experienced and highly regarded Medical Examiner. Representing the Union Flag was the young Tyro and Home Office Pathologist, Dr. Stuart Hamilton. After a bruising (but strangely hilarious) contest over hot coffee and croissants, Dr. Gunther claimed victory as the result of a technical knockout (Hamilton's inability to stand still near a microphone) while the British challenger claimed a points victory based on the fact that he had more letters and titles after his name. The conflict remained unresolved and the rivalry festered. It was unfinished business.

Until now.

In Washington, DC, these two intellectual pugilists will bring their knowledge and experience together again, honed by research and training to answer once and for all one of the great questions of our era, "Who has the finest approach to expert testimony between these two great nations?" On the one side, Dr. Gunther has the well-defined gatekeeper role enshrined in case law. On the other side, Dr. Hamilton has a two paragraph "expert witness self-certification" clause to put into his reports. In her corner, Dr. Gunther has *Daubert*, *Joiner,* and *Kumho*. In his corner, Dr. Hamilton has a week of expert witness training and a well-developed sense of his own superiority.

There is no doubt that the medicolegal investigation of death has diverged between England and the United States, but also the way that the legal systems has diverged seems even greater. Therefore, the role of the expert and the way evidence is presented in court is now substantially different. English pathologists are permitted to comment upon injuries in non-fatal cases and many Home Office pathologists provide expert reports to both the prosecution and defense (but not in the same case—that would be weird). Do these differences make one

system more likely to produce an outcome that best serves the interests of justice, or are the two approaches equally valid? Only a deep exploration of the issues (punctuated by sarcastic observations regarding "colonials" and "losing the war of independence") can answer this question.

Expert Evidence, Forensic Pathology, Jurisprudence

BS3 Working With Law Enforcement and Prosecutors: A Conversation With Two Former Feds

Timothy P. Ryan, MS, and Alan E. Brill, MBA, Kroll Advisory Services, 300 Harmon Meadow Blvd, Ste 305, Secaucus, NJ 07094; and Michael DuBose, JD*, Kroll Advisory Solutions, 2101 L St NW, Washington, DC 20037*

Educational Objective: After attending this presentation, attendees will understand how both law enforcement and prosecutors view cases involving digital and multimedia forensic evidence, having had the unique opportunity to discuss with two former Department of Justice officials how to understand the needs of law enforcement departments and prosecutors.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing private sector personnel with the opportunity to understand how best to work with law enforcement and prosecutors to pursue a criminal case and how to most effectively provide information to them.

Private sector computer and media forensic specialists who find themselves working for their employers or clients on what could reasonably be a criminal matter ultimately have to work with law enforcement and prosecutors. After all, while a U.S. private sector individual or company can directly use the civil courts and file a lawsuit; they just can't throw someone into prison.

Even where private-sector specialists have backgrounds in law enforcement, keeping up with the evolution of investigative technology and how the investigative and prosecutorial processes change in response to new laws, new court decisions and both domestic and international standards is vital. This session will provide an up-to-the-minute update.

Working with law enforcement effectively involves gaining an understanding of what information they need in today's legal environment and how their work can be presented to law enforcement and prosecutors. What are alternatives that officials think of in terms of evidence collection (digital forensic data, log files, interview notes, affidavits, etc.), and what can you expect them to ask of you?

In this session, Michael DuBose, former Chief of the Computer Crime and Intellectual Property Section of the United States Department of Justice, and Tim Ryan, former supervisor of the largest FBI Cyber Squad in the U.S. and former Acting Director of the New Jersey Regional Computer Forensic Laboratory (RCFL), will hold an open discussion with the audience that presents a unique opportunity to learn how best to work with federal investigators and prosecutors.

Attendees will learn how the two aspects of law enforcement work together and where the private sector's investigators and forensic specialists fit in. The discussion will cover how cases are brought to the attention of law enforcement or prosecutors, the kind of information that should be provided, how cases are evaluated, and what helps—and doesn't help—in seeking government assistance. Attendees will understand why government investigators may re-visit some of the work already performed to make sure that the underlying source data was properly collected and documented. Small mistakes at the start can lead to disastrous consequences later in the process.

The role of the private sector specialists as the criminal investigation proceeds, as prosecution decisions are made, and as the prosecution prepares its case, will be reviewed, along with issues like claims of bias by defense counsel and attempts to blame the victim's company. This presentation will also discuss who in a company may be the best witness. It may or may not be the IT specialist. This presentation will discuss how they evaluate impartiality, forensic/investigative experience, and litigation experience.

With ample opportunity to interact and ask questions, this session represents the opportunity to learn from leaders in the field and improve your ability to determine what is likely to be an acceptable case, what evidence to collect, how to document it, when to contact the authorities, and how to work with authorities throughout the investigative and trial process.

While both speakers are former government officials, they are not speaking as representatives of any government agency, and their views are strictly their own.

Law Enforcement, Prosecution, Cooperation

BS4 They Really Are Out to Kill You: The Inconvenience of Lawyer Involvement When Presenting Forensic Testimony

Roderick T. Kennedy, JD, Court of Appeals, PO Box 25306, Albuquerque, NM 87125-0306*

Educational Objectives: After attending this presentation, attendees will be presented with the concepts of trial examination, direct examination, cross-examination and re-direct examination and their relationship to the needs of legal adversaries. Expert testimony is seldom either complete or direct evidence of what a trial is about, but is critical to the fact-finder's understanding of the evidentiary picture. Attendees should take away from this presentation an appreciation for the need of expert and technical witnesses to understand trial preparation and procedure from the lawyer's standpoint, as well as the roles of adverse parties' representatives. This understanding should improve witnesses' work flow, documentation, and preparation for trial.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing an understanding of the goals behind lawyers' examinations of witnesses and the purposes for which their testimony is offered, while also offering tools with which witnesses can increase the effectiveness of their preparation for trial and work on the witness stand.

It seems that every time a lawyer speaks at the AAFS meeting, a forensic scientist rises from the audience to complain of mis- or maltreatment at the hands of some member of the bar. Scientists are sternly ordered to answer "yes-or-no" questions or abused by an attorney who was not held in check. Lawyers are ignorant of the principles underlying science, do not read reports, and frequently either follow a prepared script or seek testimony of a final result without any regard for the process of how it was obtained. The fear of cross-examination is universal. This presentation will discuss two propositions of value to young forensic scientists: the attorney who calls you as a witness is likely your worst enemy, *not* the one on the other side; and witnesses who are beaten up on cross-examination likely had it coming, though not necessarily because of anything they did.

Being largely a product of the legal system's need to explain or clarify other evidence at trial, the forensic sciences occupy an interesting place in the trial court. Depending on the adversary process for their living, scientists who testify must maintain a degree of objectivity, independence, and honesty that may seldom be apparent in the legal milieu. The temptations to pick sides or to act in concert with the side that employs the expert—usually the government in a criminal case—can be overwhelming, but must be avoided at all costs. The result of the work in the lab is what counts, not the result of others' work in the courtroom.

Witnesses cannot delude themselves to think they know more about trials than the lawyers do. The process of questioning a witness is designed to present a finite amount of useful information, provide some perspective as to its context or limitations, and finish. Direct examination that does not provide the proverbial "whole truth" subjects the witness to brutal cross-examination. Cross-examination by definition

consists of leading questions largely answered "yes or no," and is designed to expose any limitations or flaws in a witness's testimony. The lawyer who was not prepared to do a complete and thorough direct examination commits two sins: leaving the witness exposed to what the witness will doubtlessly feel is abuse on cross-examination, and being powerless to rehabilitate that beat-up witness on re-direct examination, which is designed to present the final evidentiary picture provided by the testimony and clear up any misconceptions about it.

The witness who is evasive, leaves out contextual information, or who is combative (whether by instruction or disposition) will likely suffer terribly on the witness stand. Some attorneys prefer witnesses to be less than forthright, but the expert has an ethical responsibility to science that includes acknowledging its limitations and uncertainties, as much as a responsibility to the witness oath "to tell the whole truth." The ethical expert should not allow an attorney to present an inaccurate or unfairly incomplete picture, but in any instance where it happens, should expect vigorous and perhaps nasty cross-examination where opposing counsel is better prepared than the witness's own side.

Trial Practice, Cross-Examination, Professionalism

BS5 How to Write Bestselling Novels and Screenplays in Your Spare Time: Tips From the Pros

Melissa Mourges, JD, and Martha Bashford, JD, New York County, District Attorney's Office, One Hogan Place, New York, NY 10013; Linda Fairstein, JD, 211 E 70th St, No 25A, New York, NY 10021; Jonathan Hayes, MD*, OCME, 520 1st Ave, New York, NY 10016; Kathleen J. Reichs, PhD*, Univ of North Carolina-Charlotte, Dept of Anthropology, Charlotte, NC 28223; Karen Bergreen, JD*, 420 E 51st St, New York, NY 10022; and Jan Burke, BA*, 11278 Los Alamitos Blvd, No 351, Los Alamitos, CA 90720*

Educational Objectives: The goal of this presentation is to give forensic scientists the background to lend accuracy and authenticity to the efforts of movie marquees, TV lineups, and best-seller lists that are filled with depictions of grisly crime scenes, futuristic crime solving techniques, and gripping courtroom encounters where drama and forensic science meet head on in the entertainment and publishing worlds. After attending this presentation, attendees will be able to: (1) give advice about how to write novels and screenplays; (2) understand how to attract agents and publishers; and, (3) understand how to market oneself using social media.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by enabling members to learn from five published authors how to turn their experience and insight into books, TV, and movie scripts and more, while also learning how to contact agents, submit manuscripts, and self-promote on social media.

Do you ever feel you are only one crime scene away from a *New York Times* bestseller? Do your friends constantly say, "You have the *coolest* job! You should write a book!" Do you find yourself muttering, "Objection!" during the courtroom scenes in *The Good Wife*? This is your chance to learn from five published authors on how to make that leap.

Linda Fairstein spent 25 years as head of the Manhattan DA's sex crimes unit, and is America's foremost legal expert on sexual assault and domestic violence. Linda's 14th novel starring prosecutor Alexandra Cooper (a thinner, blonder, and younger version of Ms. Fairstein) was released in July 2012. *New York Times* bestseller *Night Watch* is a knowing take on a rape scandal involving a hotel maid and a certain international financier known by his initials—with a surprising twist.

Jonathan Hayes is a veteran New York City forensic pathologist and author of the Jenner series of forensic thrillers, *Precious Blood, A Hard Death,* and the upcoming *Monster Park*. As a freelance writer, he's written for the *New York Times*, *GQ*, *New York Magazine*, *Food & Wine*, *Gourmet,* and *Martha Stewart Living* where he was contributing food editor. His Facebook, Twitter and Tumblr streams are renowned for their inappropriateness.

Karen Bergreen graduated from Harvard, became a lawyer, worked in a big firm, and clerked for a federal judge. None of that was as much fun as her current career as stand-up comic and author. Karen has two published novels: *Following Polly* and the recently released *Perfect is Overrated*. Karen is skilled in the art of self-promotion as publicity budgets for lesser-known authors have dried up. In between gigs on Comedy Central and NYC's Gotham and Comic Strip comedy clubs, Karen is a TV actor, but notes her range is limited: "I can't do anything that involves crying or porn."

Kathleen Reichs is a forensic anthropologist and bestselling author of the fabulous *Bones* novels about crime solver Temperance "Bones" Brennan who is, coincidentally, a forensic anthropologist. The TV show *Bones*, based on that series, is now in its eighth season. As show producer, Dr. Reichs is a stickler for scientific truth in those scripts. Her latest novel, *Bones Are Forever*, was released in August 2012.

Jan Burke has won every award there is for mystery writing, including the coveted Edgar from the Mystery Writers of America. Starting with *Goodnight, Irene*, she has crafted the 14 Irene Kelly mysteries, starring SoCal investigative reporter Irene Kelly and her husband, homicide detective Frank Harriman. Jan also heads the Crime Lab Project to raise awareness of problems facing crime labs and to fund forensic sciences.

Bestsellers, Novels, Screenplays

BS6 The Ballistic Evidence in the Assassination of John Fitzgerald Kennedy

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Educational Objectives: After attending this presentation, attendees will gain a complete understanding of the firearms evidence, the exterior and wound ballistic properties of the Model 91/38 6.5mm Carcano rifle and Winchester 6.5x52mm ammunition responsible for the assassination of John F. Kennedy (JFK) and the wounding of Texas Governor John Connally in Dealey Plaza on November 22, 1963. Such an understanding should dispel the many bizarre, fanciful, and even absurd claims and notions propounded by the numerous naive, uninformed, self-proclaimed experts, and agenda-driven individuals who know little or nothing about the exterior and wound ballistic behavior of this rifle/ammunition combination.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing attendees with new insight into the connections between Lee Harvey Oswald, the fatal rifle recovered at the sixth floor window of the Texas School Book Depository building, and the exterior and wound ballistics of this rifle and its ammunition.

With the possible exception of the 9/11 attacks on the United States, the November 22, 1963, assassination of President John F. Kennedy was the most horrific event in the last half-century of America's history. It was also one of the most documented events of that time, largely as a result of three amateur 8mm movie-makers capturing our 35th president's murder from three locations. Without question it was, and is to this day, the ultimate shooting reconstruction case.

The trail of documents, physical evidence, eyewitness accounts, and matters of motive and opportunity all led to Lee Harvey Oswald as the sole assassin.

Yet hundreds, perhaps thousands of articles, editorials, television specials, movies, and claims by would-be and self-proclaimed experts continue to offer bizarre, even absurd accounts and explanations of the November 1963 events in Dealey Plaza. Their origins are largely due to the fact that few people, even many forensic practitioners, knew very little regarding the exterior and terminal ballistics of full metal-jacketed rifle bullets. This is still the situation today and it is especially true insofar as the unusual and uncommon 6.5mm Carcano rifle and special ammunition identified as being responsible for the assassination of JFK and the wounding of Texas Governor John Connally.

This presentation will provide a detailed review of the ballistics evidence recovered at the scene and from the two victims. This will be followed by a thorough description and demonstration of the exterior and terminal ballistics of the 6.5mm Carcano rifle and ammunition found near the sixth floor window of the Texas School Book Depository building. A trace of the Model 38, 6.5mm Italian Carcano rifle, serial number C 2766 quickly revealed its mail order purchase by Oswald from Klein's Sporting Goods in Chicago, Illinois. A palm print subsequently matched to Oswald was found on the underside of the barrel in an area under the forward portion of the stock. Later investigation would reveal that Oswald had brought the disassembled rifle into the Texas School Book Depository building, where he was employed, by wrapping it in brown paper and claiming it contained curtain rods for his residence.

Techniques not available at the time or even in the three to four decades following the assassination will be employed to further explain the events in Dealey Plaza and to address those few issues and questions that have some merit, such as the shot that missed President Kennedy and Governor Connally. These techniques include computer enhancement of the Zapruder, Bell, and Nix films, 3D laser scanning and reconstruction of the entire scene, Doppler radar tracking of 6.5mm Carcano bullets over the distances involved as well as through various tissue stimulants at selected ranges, wound ballistic properties and ricochet behavior of these bullets, and the acoustics of the gunshots from such a rifle and its supersonic bullets.

JFK Assassination, Exterior Ballistics, Wound Ballistics

BS7 The Washing Away of Wrongs: Forensic Medicine in 13th-Century China

Brian McKnight, PhD, 2340 E 4th St, Tucson, AZ 85719; and Jeffrey M. Jentzen, MD*, Univ of Michigan, 300 N Ingalls, NI2D19 - SPC 5452, Ann Arbor, MI 48109*

Educational Objective: After attending this presentation, attendees will obtain an understanding of the sophistication and complexity of death investigations in 13th-century China by the author and translator of the ancient Chinese medicolegal text, *The Washing Away of Wrongs*.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by instilling an appreciation of ancient Chinese death investigation practices and bridging cross-cultural comparisons on the similarities between Chinese and European medicolegal practices.

The *Xiyuan Ji Lu* or *The Washing Away of Wrongs* was written by Song Ci (1186-1249), a jurist in 1247 C.E. during the Northern Song dynasty as a handbook for governmental officials who were assigned the task of holding inquests on homicides and other unnatural deaths. In China, state-ordered forensic investigations date back to 222-207 B.C.E. in the Ch'in dynasty. *The Washing Away of Wrongs* is earliest extant systemic treatise on forensic medicine produced in the world. Drawn from several older texts, it served as a template for subsequent Chinese works on the subject through the end of the 19th-century. In ancient times, Chinese investigators understood the importance of forensic examinations and the problems of distinguishing accidental from intentional deaths, suicide from murder, differentiating premortem from postmortem wounds, and discerning natural from unnatural death.

The most recent translation of the work was performed by Brian E. McKnight, PhD, while at the University of Michigan's Department of East Asian Studies. McKnight's masterful translation brings to life the 13th-century Chinese death investigation process. The book was "always carried to the scene of the inquest by the high territorial official." *The Washing Away of Wrongs* illuminates not only the general Chinese administrative patterns, but also the style of traditional legal practice. Song's book provides the clearest picture of the traditional Chinese detective processes. The ridged Chinese penal system required a systematic guide for death investigation. The elaborate Chinese bureaucratic system stands in contrast to the primitive system in use in Europe at the time. *The Washing Away of Wrongs* was reprinted and updated for centuries and was still in use at the time of Republican period in 1912 after some 25 editions. It was exceptional if physicians were involved in death investigation as McKnight himself observed, "the Western marriage of professional medical knowledge and forensic practice was lacking in China."[1]

Magistrates conducted inquests in China as early as 995 C.E. The actual examination of the body was carried out in the inquest by the wu-tso or "coroner," who were lowly figures, most often undertakers who might be more accurately called "coroner's assistants." Also present at the inquest was the accused, clerks, and the victim's family. There was a rule that a body could not to be moved and that the magistrate had to go to the autopsy site, accompanied by a clerk from the Bureau of Punishments. Documents used to validate the inquest were very sophisticated. By 1204, printed sketches showing the front and back of the body were used. With the exception of cutting flesh to examine bones, Chinese forensic examinations concentrated on the external examination of victims.[2]

Song's guidebook described methods to look for in hanging deaths, gave examples of different forms of strangulation, as well as the observation of bruises, self-inflicted wounds, and discussions of internal injury. As a type of casebook, *The Washing Away of Wrongs* provided cases for the magistrate and clerks to learn from in their own practice. The text discusses the use of bones and their color in the diagnosis of poisoning. Experimental manipulations were also a part of the text. The presence of ashes in the mouth of a pig used to ascertain whether an individual was alive or dead prior to being consumed with fire. Red cloth was used to evaluate bruises not visible to the naked eye. *The Washing Away of Wrongs* provides an ancient landmark from which to measure our own progress in medicolegal death investigation.

References:

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2. Pierre-Étienne Will, "Developing forensic knowledge through case in the Qing dynasty," in *Thinking About Cases: Specialist knowledge in Chinese cultural history*, eds., Charlotte Furth, Judith T. Zeitlin and Ping-chen Hsiung, (Honolulu: University of Hawaii Press, 2007).

China, Legal Medicine, History

BS8 Thomas Krauss Memorial Bitemark Breakfast — Lessons From Eyewitness Identification Research for Forensic Scientists

Barry C. Scheck, JD, and Karen A. Newirth, JD, The Innocence Project, 40 Worth St, Ste 701, New York, NY 10013*

Educational Objective: After attending this presentation, attendees will understand how research into the causes of eyewitness misidentification can improve the collection and evaluation of forensic evidence.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by explaining aspects of social science research on eyewitness identification that relate to the work of forensic scientists. Attendees will be able to consider research on relative judgment and suggestive feedback and how this research can improve the work of forensic scientists.

Eyewitness misidentification is the most common contributing cause of wrongful convictions, occurring in nearly 75% of the 297 DNA-based exonerations nationwide. For more than 30 years, psychological scientists have studied perception and memory as applied to eyewitness identification and have produced a robust body of peer-reviewed scientific research that the New Jersey Supreme Court recently declared to be the "gold standard in terms of the applicability of social science research to the law."[1] The scientific research has resulted in clear recommendations for police procedures that will result in more reliable and accurate identifications and will reduce the likelihood of eyewitness

misidentification. The underlying research and the recommendations are useful when contemplating how to improve the evaluation of forensic evidence.

In formulating recommendations for police practices, scientists studying eyewitness identification have considered the relationship between relative judgment and misidentification and have concluded that when witnesses rely upon relative judgment (comparing members of a lineup and choosing the member who looks most like the memory of the perpetrator), they are more likely to make errors than when they rely upon absolute judgment (making a decision about each lineup member independent of the other lineup members). Scientists have theorized that the use of relative judgment results in more errors because some members of the lineup will always look more like the perpetrator than the others, whether or not that lineup member is actually the perpetrator. Using procedures that force witnesses to make absolute judgments about each lineup member prevents them from comparing lineup members so that they don't select the "best match" among the group. Instead, it asks that witnesses make an independent evaluation of each lineup member against the memory of the perpetrator.

Scientists studying eyewitness identification have also focused on the effects of confirming feedback by administrators on eyewitnesses and have discovered that confirming feedback of eyewitness identifications can dramatically affect eyewitness reports on a variety of measures, including eyewitness's reports of the original viewing experience, of the lineup viewing experience, and of their confidence and certainty in their identifications. In all cases, confirming feedback dramatically increased the positive reports of witnesses on all measures.

Eyewitness identification research on relative judgment and confirming feedback have important implications for forensic analysts who can be equally affected by relative judgment and the influence of external information. The problem of relative judgment in the context of fingerprint analysis was addressed in the Office of the Inspector General Report on the Brandon Mayfield Case, which recommended practices to protect against relative judgment, including linear analysis.[2] Researchers, including Itiel Dror and his colleagues are working to understand the potential influence of external information on forensic analysts.[3] Dror *et al.* have performed several groundbreaking studies that examine how external information affects the behavior of experienced fingerprint analysts.[4]

References:

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3. *See, e.g.,* Dror, I. E. (2012). Expectations, contextual information, and other cognitive influences in forensic laboratories. Science and Justice, 52 (2), 132.
4. *See, e.g.,* Dror I. E., Champod C, Langenburg G, Charlton D, Hunt H, Rosenthal R. Cognitive issues in fingerprint analysis: inter- and intra-expert consistency and the effect of a "target" comparison. Forensic Sci Int 2011; 208:10–7.

Eyewitness, Identification, Bias

L1 Ensuring Continuing Access to Our Nation's Heritage: The National Archives' John F. Kennedy Assassination Records Collection

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Educational Objectives: After attending this presentation, attendees will understand the National Archives and Records Administrations' historic and ongoing role in accessioning, organizing, preserving, documenting, and making accessible the records and artifacts contained in the John F. Kennedy Assassination Records Collection.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a practical foundation for the ongoing discussion of current and proposed further scientific research into the JFK assassination as the 50th anniversary of the tragedy approaches.

John F. Kennedy was killed on November 22, 1963. Almost 30 years later, hoping to allay lingering doubts about the circumstances surrounding that event, Congress enacted the President John F. Kennedy Assassination Records Collection Act (Public Law 102-526, signed on October 26, 1992, by President George H.W. Bush). One provision of the law mandated that all assassination-related material be housed in a single collection in the National Archives and Records Administration (NARA). The clear intent of the law was to open most of the records for research. On December 28, 1992, NARA established the John F. Kennedy Assassination Records Collection, which now contains more than five million pages of assassination-related records, photographs, motion pictures, sound recordings, and artifacts.

The John F. Kennedy Assassination Records Collection includes federal government records of presidential commissions, such as the Warren Commission; congressional committees, such as the House Select Committee on Assassinations; executive branch agencies, such as the Federal Bureau of Investigation and the Central Intelligence Agency; and judicial branch courts, as well as a variety of donated historical materials.

The President John F. Kennedy Assassination Records Collection Act defined narrow categories of information whose release could be postponed and established the Assassination Records Review Board (ARRB) to consider all individual agency decisions to postpone the release of records. The Act requires that all assassination-related records be opened by 2017, with the exception of documents certified for continued postponement by the President. Additions to the John F. Kennedy Assassination Records Collection holdings are made as agencies continue to review records identified as relevant and transfer newly opened records to the National Archives.

This presentation will provide an overview of the John F. Kennedy Assassination Records Collection holdings at the National Archives and Records Administration and a review of current search-and-access information and procedures for potential researchers. The presentation will also illustrate the ongoing preservation and documentation efforts undertaken by the National Archives since enactment of the President John F. Kennedy Assassination Records Collection Act, during and after the Assassination Records Review Board tenure. These efforts include the development of preservation strategies for the Zapruder film and for the Dallas Police Department dictation belts; further scientific examinations brokered for one of the bullet fragments and its associated trace evidence, including the microscopic identification and characterization of trace fibers and human tissue; the design and fabrication by the NARA conservation laboratory of custom-built housings for the long-term preservation of assassination-related artifacts and associated samples; and, the currently proposed high-resolution imaging of bullets and bullet fragments from the Warren Commission exhibits.

JFK, Assassination, Records

L2 Does Your Family Have the Munchies? It Might Be By Proxy

Janean M. Fossum, BSN, PO Box 11053, Eugene, OR 97440; and Melodie Brooks, MSN*, 3091 Widdock St, Erie, MI 48133*

Educational Objectives: After attending this presentation, attendees will: (1) understand the principles of medical child abuse, including a brief historical review, distinguish Munchausen By Proxy (MBP) from Munchausen Syndrome; and, (2) recognize victim and offender characteristics, and comprehend the importance of a Multidisciplinary Team (MDT) approach for successful investigation and prosecution. The use of case studies will provide examples of the multiple populations affected and often a diagnosis of exclusion is needed as evidence to validate this form of child maltreatment.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by explaining the principles of medical child abuse, concluding with a clear understanding of the implications to health care providers, law enforcement, and protective services. A review of victim and offender characteristics, and the importance of the multidisciplinary team will also be discussed. This presentation will provide an overall view of the magnitude and covert nature of MPB. Moreover identifying how coordinated forensic investigations can impact children's lives and the communities in which they live.

The term Munchausen was coined after an 18th-century German dignitary Karl Friedrich Hieronymus Freiherr von Munchausen. The Baron was well known for his eccentric tales that many times proved to be fictitious. Moreover, the Munchausen term was first used in 1951 by Dr. Richard Asher who described self-induced illnesses in his patients.[1]

Today, many terms have come to symbolize Munchausen Syndrome, including fabricated illness and hospital-addiction syndrome. Additionally, this disorder is classified as a psychiatric illness, mainly noted in men and believed to bring individuals gratification from the sympathy and attention given to the individual by medical personnel; however, another form of this attention seeking behavior is known as MBP.[1]

MBP or factitious disorder, unlike Munchausen's, is defined as a form of medical child abuse hallmarked by a parent, mainly mothers, over reporting illness or creating a venue where children have unnecessary medical procedures performed.[2] This term was first identified in 1977 by R. Meadow, MD. He noted that Munchausen Syndrome was present in two of his patients' mothers who had projected fabricated illnesses onto their children. The literature describes two roles played by perpetrators of this abuse, inducers and fabricators. Inducers directly cause illness with the child and fabricators exaggerate their child's symptoms to receive attention.[1]

The incidence of MBP is as elusive as the perpetrators of this form of child maltreatment. The literature suggests that a minimum of 600 new cases a year are noted from suffocation and non-accidental poisoning in children. Two main contributing factors for the unknown incidence is a

lack of a standard definition for MBP and a central repository for data related to this phenomenon. Moreover, a collective approach is required to investigate and expose this form of child abuse.[3]

The coordination of multiple disciplines is required across agencies in order to be successful with investigation and prosecutorial efforts with MBP. Each discipline must focus on its own specialty but communicate openly with other team members. Step one includes awareness of the disorder which may include unexplained medical illnesses after testing or a history of multiple visits to different hospitals and physicians. Step two involves a complex organized MDT with a goal of complete removal of the child from the offending caregiver. A planned hospitalization is recommended with hospital video surveillance as an element required to produce evidence of deliberate harm to the child. Additionally, a thorough and complete medical record review should be conducted.[3]

References:

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3. State of Michigan Department of Human Services (2007, June). Munchausen by proxy: A collaborative approach to investigation, assessment and treatment. Retrieved July 1, 2012, from website: http://www.michigan.gov/documents/dhs/DHS_PUB_0017_200457_7.pdf.

Medical Child Abuse, Forensic Nurse, MDT

W1 Practice, Procedures, and Protocols: How SWGDE, SWGIT, and FISWG Can Help You Navigate the Complex World of Digital and Multimedia Evidence

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After attending this workshop, attendees will: (1) become familiar with forensic guidelines for the proper recovery, processing, storage and authentication of digital and multimedia evidence (DME); (2) understand strategies regarding admissibility of DME in court; and, (3) learn information regarding current research and educational requirements in DME.

This workshop will impact the forensic science community by increasing awareness regarding digital and multimedia evidence and the proper procedures associated with it. With this, attendees will be able to utilize this type of evidence, and to ensure to the greatest extent possible that the justice system and the public benefits from it.

With today's technology more DME evidence may find its way into the courtroom. Analyses conducted on computer files, electronic devices, images, videos, and audio recordings may be crucial to an investigation. The increased use of DME has led some to raise criticisms in an attempt to exclude such evidence. It is critical that agencies and investigators take measures to ensure that the technologies, practices, and procedures used can be defended in the court room. Recognizing this challenge, multiple Scientific Working Groups have been formed over the years, starting with the Scientific Working Group on Imaging Technology (SWGIT) in 1997 and the Scientific Working Group on Digital Evidence (SWGDE) in 1998. In 2009 the Facial Identification Scientific Working Group (FISWG) was formed. Together these Scientific Working Groups ("SWGs") address the myriad of issues that can arise related to the science and technology of digital and multimedia evidence.

As of June 2012, SWGDE, SWGIT, and FISWG have published over fifty documents that address issues ranging from chain of custody and the proper preservation of digital and multimedia evidence, to procedures for video and image processing, best practices for mobile phone examination, image authentication, maintaining the integrity of DME, guidance on procurement and deployment of face recognition systems, and recommendations for training in DME procedures.

This workshop will familiarize investigative, forensic, and courtroom personnel with these documents and the guidance contained therein, so that the attendees can incorporate them into the procedures within their own practices. Attendees will learn about chain of custody issues as they relate to recovery of DME at crime scenes, including proper recovery of computers as well as crime scene photographs and surveillance video recordings from both analog and digital systems. Attendees will also receive guidance regarding proper procedures for preserving such data and ensuring its integrity, as well as learn where they can go to find out more about it.

Once evidence has been acquired in an investigation, it must be processed and analyzed to produce meaningful results. SWGDE, SWGIT and FISWG have all developed guidelines regarding what sort of processing steps or approaches may be most useful in a forensic setting and often provide guidance on how to document those steps. Included in such best practices are those to ensure the integrity of the evidence, as well as demonstrate the validity of the analytical procedures used. Workshop attendees will learn about the options available to them for ensuring such integrity, the difference between integrity and authentication, and preserving their data over the long run.

Likewise, many in forensic science remain concerned regarding the admissibility of DME within the courtroom. This workshop will provide attendees with a reference list of case law, as well as describe some myths and misconceptions associated with DME. These documents should help them ensure that their evidence is admitted in court.

Finally, in keeping with the theme of this year's meeting, "*The Forensic Sciences: Founded on Observation and Experience, Improved by Education and Research*," attendees will receive an overview of many of the most recent research publications related to DME. This will demonstrate the active research being performed in the discipline and should help attendees identify future avenues of research.

Digital Evidence, Audio/Video/Image, Face Recognition

W2 Electrocution, Electrical Injury, and Lightning Death Investigations

Ronald K. Wright, MD, JD, 1000 Ducks Nest Rd, Turtletown, TN 37391; and Helmut G. Brosz, PEng, BASc*, Institute of Electro-Pathology, Inc., 64 Bullock Dr, Markham, ON L3P 3P2, CANADA*

After attending this presentation, attendees will be able to: (1) identify both common and uncommon electrical low and high voltage circuits having the potential to produce electrical shock, injury or death; (2) properly document (photograph and draw) electrical injury defects; (3) properly document the scene of an electrical injury accident; and, (4) differentiate between lightning and non-lightning electrical injury.

This workshop will impact the forensic science community by equipping attendees with theoretical and practical knowledge to effectively investigate electrical injuries and deaths. The workshop will examine the concept of Ohm's Law and its application to case investigation. Included in this discussion will be the use of Ohm's law to estimate the current flow when the voltage is known.

Direct and alternating current and the differences in lethality and injury will be discussed along with the history of the dispute between George Westinghouse and Thomas Edison over the distribution of electrical current. As a corollary, an explanation of the electric chair will be explored, which was a spinoff of their dispute. Professor Dalziel's work on perception thresholds and no let-go thresholds in humans will be explored. Included in the presentation will be a chart showing the effects of current flow on humans at varying current amounts.

The stereotypic automatic response to current flows at or above let-go threshold will be examined, with case examples presented, including the differences between upper and lower extremities to the flow of electricity. The delay between repolarization and muscle action will be investigated and the effects of these phenomena upon case work explored. The amount of time required to lose consciousness following the onset of ventricular fibrillation will be elucidated and the reasons for this explored. The effect of this time lag upon civil litigation will be discussed and the varying interpretations of different state courts will be explained.

Examination of the effects of both high and low voltage exposure in humans will be presented. The biphasic response to current flows below and above one ampere will be explained, including the length of time required to initiate ventricular fibrillation. The concept of cellular poration, which is seen in high voltage electrical injury, will be discussed including the effects of poration upon the human body and the devastating tissue destruction which ensues.

Special consideration of lightning and other extremely high voltages will be shown, including the effects of explosive expansion of gases and

the explosive effects upon ear drums and clothing. In the case of both lightning and distribution lines around 250,000 volts, if a person is near or in the circuit, the person's clothing is explosively torn and the ear drums ruptured from the explosion. The magnetization of ferrous metal objects in or near a lightning strike will be examined. The importance of lightning in explained death or injury has significance in the civil litigation arena, as generally lightning, as an "act of God," is not compensable.

Case examples from the experiences of Professor Brosz and Dr. Wright will be examined and utilized to illuminate the approaches to all deaths and injuries which may be from electrical current whether manmade or natural. Special consideration of the possibility of both over diagnosis and under diagnosis will be examined.

Electrocution, Electrical Injury, Lightning Deaths

W3 Analysis and Interpretation of Chemical Unknowns

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After attending this workshop, attendees will better understand the analysis of various types of chemical unknowns that frequently are encountered in the forensic laboratory.

This workshop will impact the forensic science community by providing much-needed education in a discipline that currently is lacking in substantial formal training.

Chemical unknowns present a unique challenge to forensic laboratories. As the name suggests, these are materials of an unknown origin and composition that may be found in connection with a virtually limitless number of scenarios of forensic significance. In the best case scenarios, they are common single component materials that have benign uses. At worst, they are complex mixtures of rare, dangerous compounds that have the potential to cause great harm.

Chemical unknowns are submitted to forensic laboratories for a variety of reasons, including: (1) outright identification of a material, commonly, either a suspicious powder or liquid in a public space or a white powder included in a threatening letter; (2) analysis of an item to determine if any tampering may have occurred, commonly in the form of an allegedly adulterated foodstuff; or, (3) comparison of an unknown material found on or in the possession of a suspect to a similar material recovered from a crime scene. An example of the latter might be a white powder observed on the body of a homicide victim being compared with lime from the garage of a suspect. The information obtained from the analysis and characterization of these unknown substances can be used to help associate suspects with scenes and victims as well as to provide information for investigative purposes.

A wide variety of unknown materials are routinely encountered as chemical unknown evidence, to include solids, liquids, gases, and both homogeneous and heterogeneous mixtures of these various phases in any combination. The specific phases present may be organic, inorganic, or mixtures thereof, and may come from either natural or man-made sources. Samples that occur in different physical states will be amenable to different analytical approaches, and examiners should ideally have access to a wide variety of instrumentation and be intimately familiar with the strengths and limitations of each piece of equipment.

Considering the broad range of scenarios and materials that could be encountered, the analysis and characterization of chemical unknowns can pose some serious challenges to the forensic scientist. With this in mind, the primary goal of this workshop is to educate practitioners, educators, and other interested members of the forensic community about the types of approaches that have proven successful for the presenters. The presentations will provide some guidance on a logical approach for handling such cases. The presenters will include individuals with diverse backgrounds and experience in the identification of chemical unknowns. This workshop will outline the techniques that are commonly used for the characterization, identification, and comparison of chemical unknowns. Specific attention will be paid to sample preparation techniques, microscopical examinations, and various forms of instrumental analysis, to include Fourier Transform-infrared spectroscopy, Raman spectroscopy, scanning electron microscopy, energy dispersive spectroscopy, X-ray fluorescence, X-ray diffraction, chromatographic techniques, and mass spectrometry. During the course of the workshop, presentations covering safety, sample handling, interpretation of results, and reporting strategies will be provided. In addition, the attendees will be given handouts listing a wide variety of published literature, online resources, training opportunities, and more that cover instrumental theory, application, and interpretation. Several of these resources have proved invaluable to the presenters for the purposes of determining whether the identified materials in a chemical unknown are consistent with a specific household product, for example.

In order to better appreciate many of the points made during the lecture portion of the workshop, the attendees will have the opportunity to perform hands-on exercises designed to familiarize them with some of the techniques that have been discussed. These hands-on activities will include actual examination of chemical unknowns, with an opportunity to perform sample preparation and microscopical examination of the samples, with instrumental results provided by the presenters to assist in their interpretation of the samples.

Chemical Unknowns, Trace Evidence, Microscopy

W4 Signature Examination of Healthy and Impaired Writers

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After attending this presentation, attendees will: (1) become more aware of the kinematic values of strokes in static signatures; and, (2) be exposed to features of signatures that are impacted by the effects of age, illness, and substance abuse.

This presentation will impact the forensic science community by increasing the knowledge base of forensic document examiners in the area of kinematic analysis of the signatures of healthy writers and those affected by age, illness, or substance abuse.

The examination of signatures is the most prevalent type of casework for many Forensic Document Examiners (FDEs). In many cases, a person's signature may change over time in subtle ways increasing the difficulty in estimating authenticity. Among several considerations, the FDE may take into account the age and health of the writer and changes in health status. Illness or impairment in a writer may cause that writer's signatures to exhibit a greater range of variation than that of a healthy writer. Since the FDE has to make determinations about whether features in a signature are the result of variation (authenticity) or a fundamental difference (non-authentic), the presence of an illness or impairment that affects handwriting can make that determination quite difficult.

In this workshop, participants will have an opportunity to examine genuine and simulated signatures of healthy and impaired writers in two hands-on sessions. Didactic sessions will provide participants with the necessary empirical and scientific bases to evaluate questioned signatures. Participants will evaluate signatures prior to and then after the didactic sessions.

The morning didactic lecture will introduce to participants principles of motor control and motor programming such as motor equivalency. This lecture will provide a framework for the first hands-on session where participants will assess the authenticity of questioned signatures of healthy writers written under various conditions. A second lecture will be given on the topic of stroke kinematics. Kinematic analyses provide descriptions of how strokes are produced in space and time. Size, velocity, and acceleration are important in kinematics and are often

parameters which are inferred by FDEs in the examination of static images of handwriting. The kinematics involved in the production of a signature may be different depending on whether the signature is authentic or forged. Additional variation may result due to differing conditions in which the signature was executed. For example, writing when standing may produce different kinematic results than writing when sitting.

This lecture will be followed by a hands-on session to develop skills for detecting invariant features in signatures written under various spatial constraints. Participants will be tasked with examining specific features in static signatures and evaluating their kinematic values. The results will be correlated with the actual kinematic values. From this feedback, the attendees will get an idea of their individual ability to estimate kinematic values from static signatures. Additionally, participants will be asked to examine pairs of signatures and determine whether specific kinematic parameters are useful in distinguishing one signature from another. A general discussion will follow about the pros and cons of the current and kinematic approaches in detecting forged or disguised signatures.

In the afternoon sessions, participants will assess more challenging questioned signatures based on knowledge of how advanced age, dementia, and substance abuse impact handwriting. The session will begin with an overview of signature and handwriting formation in young and aged healthy adults, individuals with known histories of illegal substance use, and individuals with dementia. Data will be presented on cross-sectional age-related changes in signature kinematics demonstrating nonlinear changes in stroke amplitude, duration, stroke velocity and smoothness to address questions pertaining to the presence of Parkinsonian handwriting features in the elderly writer. The lecture will include recent findings on the relationship between signature formation and cognitive status in patients with Alzheimer's disease. Participants will examine challenging signatures from writers with dementia and assess authenticity in the afternoon hands-on session. Participants will be debriefed on the results of their judgments of authenticity in relation to kinematic findings that distinguished known from questioned signatures.

The workshop will conclude with a discussion of the extent to which empirical findings increase the objectivity of the examinations. At the end of the workshop, attendees should have an increased knowledge of the neuroscientific bases of healthy and impaired writing. Additionally they will be exposed to techniques that may increase the objectivity of forensic signature examinations.

Healthy Writing, Impaired Writing, Signature Exam

W5 Science in the Courtroom: A Matter of Perspective?

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After attending this workshop, attendees will be able to: (1) understand the role, objective, and meaning of scientific evidence in the criminal justice system; (2) learn common difficulties experienced in the use and application of scientific evidence in the courtroom; (3) foster understanding between legal participants; (4) facilitate better communication, increased trust, and more productive working relationships; (5) demonstrate how this can lead to improved forensic and courtroom practice; and, (6) explain why and how this can lead to more just and reliable outcomes.

This workshop will impact the forensic science community by improving forensic and courtroom practices to achieve more just and reliable outcomes.

The need for reforms in the forensic sciences became manifested in the wake of the 2009 Report issued by the National Academy of Sciences, *Strengthening Forensic Science in the United States: A Path Forward*. One of the areas of concern touched upon was the effect of contextual bias due to the fact that most forensic labs and practitioners are either part of law enforcement or prosecutorial offices or closely aligned therewith. However, contextual issues impacting the performance, use and presentation of forensic evidence are not limited to forensic scientists. Prosecuting and defense attorney, as well as judges, also utilize and consider scientific evidence from unique perspectives that may determine what and how scientific evidence is generated and presented as well the conclusions that may be drawn from it. If forensic science is to live up its promise as a tool for the determination of truth in the courtroom, then the roles of each of the participants in a criminal case must be properly understood so that contextual issues don't undermine it. This workshop addresses forensic science, its role, objective, meaning and actual use/practice in the criminal justice system from the perspective of the system's primary participants in this endeavor, the forensic scientist, judge, and prosecuting and defense attorney. The intended audience includes judges, criminal defense and prosecuting attorneys, and forensic scientists.

The workshop consists of three sessions. The workshop's first session will consist of each a forensic scientist, judge, prosecuting and defense attorney discussing the proper role, objective, and meaning of scientific evidence in the criminal justice system and point out how this is often different from how these are commonly viewed. Each will also discuss the difficulties encountered in trying to adhere to proper practices, in particular, those caused by the other participants in either properly or improperly trying to perform their role.

The workshop's second session will consist of live examination and cross-examination of a forensic witness in front of a judge in order to illustrate some of the issues discussed in session one. These will be accompanied by commentary pointing out issues as they arise, how the performance either does or does not conform to the properly defined practices, where difficulties arise and how they might be addressed.

The workshop's third and final session will consist of a panel discussion tying everything together for the audience. The discussion will address the unique perspectives of each of the participants, whether there are aspects of the adversarial approach that make it inherently difficult to adhere to proper practices and why and how appreciating how each role is viewed by its protagonist can facilitate better forensic and professional practices by each leading to more just and reliable outcomes. Audience participation in the discussion will be facilitated by a moderator who will keep the discussion directed towards the goals of the workshop.

Science, Courtroom, Perspective

W6 Beyond the Numbers: An Objective Approach to Forensic Toxicological Interpretation

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After attending this presentation, attendees will understand: (1) interpretative differences between antemortem and postmortem toxicological results; (2) how death scene investigation, basic forensic pathological findings, and postmortem artifacts can aid in toxicological interpretation; (3) how anecdotal observations, standard field sobriety tests (SFST), and drug recognition examinations (DRE) can enhance proper interpretation; (4) pharmacological tolerance and potential impact on interpretation; (5) how genetic differences can affect drug

metabolism; and, (6) how to objectively explore and apply literature references to case data.

This presentation will impact the forensic science community by equipping forensic toxicologists, pathologists, and other persons charged with toxicological interpretation in understanding and evaluating evidence beyond the drug concentrations alone. Additionally, this presentation will equip attendees with the knowledge and tools to objectively assess all the evidence in its full context when interpreting a toxicological result.

Forensic toxicologists, forensic pathologists, and other investigators are often tasked with the interpretation of drug, alcohol, or other xenobiotic concentrations in bodily fluids and tissues. Such requests are often made without providing the investigator with a complete contextual reference or suitable history of the case under consideration. Due to the many factors that must be considered, the interpretation of a toxicological result is most appropriately performed while considering the complete context of the result and the circumstances and history of the case. A primary context for consideration is whether the analyzed specimen is an antemortem or postmortem specimen.

In antemortem interpretation, important considerations are: the specimen analyzed (i.e., serum, plasma, or whole blood); the type of laboratory performing the analysis; the method in which the specimen was analyzed (i.e., enzymatic alcohol vs. headspace alcohol); potential pharmacological tolerance of the subject; duration and amount of drug use; clinical history; elapsed time between observed behavior and specimen collection; the results of standardized field sobriety tests and drug recognition examinations; observations of driving or other behavior, statements made at the scene; prescription information; and, potential idiosyncratic anomalies in metabolism, among others.

In postmortem interpretation, important considerations are: potential for postmortem redistribution; site and method of specimen collection; potential pharmacological tolerance; clinical history; observed pathology and postmortem artifacts (i.e., track marks, visceral congestion, pulmonary edema, skin coloration, chemical odors, etc.); notes left at the scene; missing medications; prescription history; circumstances surrounding the death (i.e., trauma, drowning, fire, etc.), the state of decomposition, postmortem interval; and, pharmacogenomic considerations, among others. Furthermore, an investigator should consider whether the collection and analysis of alternate or additional specimens such as liver, bile, vitreous humor, or gastric contents may clarify interpretative issues.

Additionally, forensic toxicologists, pathologists, and others often misuse or misinterpret published drug and xenobiotic concentrations by not considering or even recognizing the difference in postmortem and antemortem concentrations, the difference between whole blood and serum/plasma drug concentrations, the way in which therapeutic drug concentrations are determined, and failing to recognize the limitations of published data. Among the limitations of published data are potential selection biases in epidemiological studies of drugs and driving, such as those derived from drivers stopped for aberrant driving behavior, and the potential statistical biases of published "lethal" drug concentrations brought about by mathematical skewing.

Toxicology, Interpretation, Context

W7 Practical Homicide and Medicolegal Death Investigation: Practical and Clinical Perspectives Regarding the Homicide Investigation and the Medical Examiner's Determination in Various Modalities of Death Including Child Deaths and Suicide

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After attending this presentation, attendees will be able to: (1) understand the role of fantasy in sex-related death; (2) collect and preserve physical and psychological evidence in sex-related incidents; (3) determine the *modus operandi* and signature characteristics in crime scenes; and, (4) understand investigative and behavioral analysis in criminal profiling.

This presentation will impact the forensic science community by informing the audience of the dynamics and proper procedures in the investigation of sex-related homicides and death investigations.

The professional investigation of homicides and sudden violent deaths require the practitioner to employ revolutionary forensic techniques as well as the modern investigative procedures found in the "Best Practice" model of Practical Homicide Investigation®, which has become the recognized protocol for professional death investigation. In addition, straightforward and candid communication between investigators and the various forensic specialists is vital. Specifically, the medical examiner and how the determination of the cause, manner, and mechanism of death are the basis of a thorough medicolegal investigation

This workshop will familiarize forensic scientists and investigators in the art and science of homicide and death investigation. The workshop will focus on the elements of detective work and the dynamics of sudden and violent death, and provide the most practical and conventional information available to detectives and practitioners responsible for conducting intelligent investigations of violent and sudden death.

Upon completion of this workshop, participants will have gained an understanding of the practice and theory of professional homicide and medicolegal investigation. Participants will have the most current information and knowledge on various modalities of death and some of the myths of asphyxiation will be dispelled. Based on the extensive experience and education of the three presenters the forensic community will benefit and be better informed of the dynamics and proper procedures employed in the investigation of homicides and death investigations.

In the morning segment, comprehensive discussion will be provided of the practice and theories of professional homicide investigation and the preliminary duties of the death investigator at the homicide scene. In order to conduct an efficient and effective investigation, the detective first concentrates on the mechanical aspects of the death, i.e., motives and methods, wound structures, crime scene reconstruction, bloodstain pattern analysis, the cause, manner and time of death, as well as other factors that provide clues to the dynamics of the event. The detective then accesses various sources, which can be applied to his or her investigation.

The medical examiner/coroner's office is primarily concerned with the investigation of violent, sudden, unexpected, and suspicious deaths. The procedures used in the official medicolegal investigation of death fall under the supervision of the chief medical examiner or coroner, who is responsible for the evaluation and interpretation of the results of this inquiry. Medicolegal investigation of unnatural deaths, emphasizing the importance of the multidisciplinary investigation of homicides as well as suspicious and equivocal deaths will be discussed. Specifics include medicolegal death investigative systems, time of death and firearm injuries to include a discussion of gunshot wounds and the classification of gunshot wound structure.

In the afternoon segment the investigation of asphyxial deaths based on the research studies of the Working Group on Human Asphyxia will be presented. Several myths on asphyxial deaths are deeply rooted in the forensic community these myths as well as others will be discussed and eradicated. In addition there will be a discussion on blunt and sharp force trauma with a presentation of some research data on criteria to distinguish falls from blows in the challenging cases of blunt head trauma where there are no witnesses to the injury. This segment will be followed by a discussion on the investigation of suspected child homicides and child abuse-related injuries are presented as well as information on the medicolegal investigation. The workshop will conclude with a discussion on the investigation of suicide and equivocal death.

Each of the presenters bring years of practical experience and research into various aspects of homicide and medicolegal death investigation and will share their expertise with the attendees. The overall goal will be to provide comprehensive and practical information

which will serve an investigative guide in the investigation of sudden and violent death inquiries.

Homicide Investigation, Medicolegal Death, Asphyxia-Suicide

W8 Multidisciplinary Approaches to Effective Communication and Report Writing

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The goal of this workshop is to discuss the importance of communication among the various agencies involved in an investigation and the essentials of effective report writing.

This workshop will impact the forensic science community by providing encouragement, tools, resources, and support needed to give forensic professionals the ability to positively contribute to the forensic science field through effective, thorough, and clear communication.

With continual advancements in technology, various evidence collection methods, and the wide range of forensic disciplines, the importance of effective and efficient communication and the significance of accurate report writing have increased. Practitioners must communicate with individuals assigned to a case to ensure that accurate information is relayed and that evidence handling is coordinated between forensic disciplines. Analysts must keep abreast of new techniques; all the while contemplating how those findings should be presented to outside agencies and the judicial system. All of this work and coordination must then be summarized into a clear and concise report that can be used in court. The investigation of a criminal case is a constant give and take of information and this workshop will help illustrate the fact that no discipline is an island and without teamwork justice cannot and will not prevail.

Collaboration is most apparent in disciplines which require coordination with outside agencies in order to issue findings, including determining manner of death. This workshop will allow those who speak for the dead to share how to speak with them. Tanisha V. Henson will make a case that the information from law enforcement agencies can not only impact a ruling on manner of death, but can also determine if the medical examiner's office should be involved at all. Forensic Pathologist Kim Collins will discuss the pertinent information that is needed when she is working "alone in the basement" to determine cause of death.

The need for effective communication of course is not limited to the medical examiner's office. Members of the crime lab require the same exchange of information, often times with people in the same building. Ryan Rezzelle will discuss the various issues that arise as evidence moves through a crime lab and Jayne Thatcher will share the best way to communicate with the local toxicologist. Providing a managerial perspective, Jill Spriggs, lab director and current ASCLD-LAB president, will help workshop attendees define successful communication.

Of course the end result of most analyses is a report and tailoring reports to particular audiences can sometimes be difficult, as Donna Boyd will discuss from a forensic anthropology perspective. All of the speakers, however, will discuss what is included in standard reports and how they determine what goes into their report. Ted Hunt and Pam King will then discuss how these reports are used in the jurisprudence system from the perspective of the prosecution and defense, respectively.

Because true communication cannot occur without a certain amount of discourse, the end of the workshop will consist of a panel discussion with the speakers. The panel will be presented with topics important to the forensic community as well as points that arise during their presentations. Attendees will also be given the opportunity to pose questions, which will allow for a direct exchange of information.

This workshop will demonstrate that forensic analysis does not occur in a vacuum, but rather requires the work of multiple disciplines to form a complete and accurate picture of an event. This picture is painted with clear and concise reports, but only after an exchange of accurate information provides a rough sketch from which to begin. Only through open and effective communication, can a case, and ultimately a forensic discipline, progress, and move forward.

Forensics, Communications, Report Writing

W9 The Predator Next Door: Everything You Always Wanted to Know About Sex Offenders and Had No One to Ask!

Vivian Shnaidman, MD, 812 State Rd, Ste 220, Princeton, NJ 08540; Cinzia Gimelli, PsyD, PhD*, Viale Montegrappa, 29/C, Reggio Emilia, ITALY; Luciano Garofano, PhD*, Via G. D'Annunzio n.9, Parma, ITALY; Roberto Testi, MD, PhD*, Via Lessona 54/12, Torino, ITALY; Melania Lugli, PhD*, Viale Montegrappa, 29/C, Reggio Emilia, ITALY; Marina Genis, MA*, 21 Sunset Ave, Unit 149, Johannesburg, SOUTH AFRICA; Eleanor B. Vo, MD*, 285 Durham Ave, Bldg 6, Ste 2A, South Plainfield, NJ 07080-2546; and Michael Shnaidman, PhD*, 6 York Dr, Princeton, NJ 08540*

After attending this presentation, participants will: (1) understand the different types of rapists, pedophiles, and sexual psychopaths; (2) obtain a basic understanding of risk assessment; and, (3) have a clear picture of the warning signs when an individual they encounter might have a history of sexual offenses.

This presentation will impact the forensic science community by: (1) describing and differentiating the various types of sexual offenders; (2) explaining risk factors so practitioners will be able to appropriately identify and assess sexual predators; and, (3) providing information on determining risk factors in predators in order to make predictions about repeat activity.

Forensic evaluators will be able to utilize statistical and actuarial information appropriately, as well as understand the difference between a true clinical evaluation and clinical evaluations that the literature derides. Many individuals who work in the mental health field obtain the majority of their understanding about sex offenders from the popular media. This presentation will teach evaluators how to distinguish between fact and fiction. The "abuse excuse" and the politically incorrect topics of mentally ill and mentally retarded offenders will be presented and explored.

This workshop will present the following topics in as much detail as time permits:

- What might qualify as a sex offense can be surprising. Some of the theoretical underpinnings from the psychoanalytic literature will be touched upon, but in a very understandable and accessible manner.
- What is a sexually violent predator? Legal and colloquial understanding of this term differs greatly and in order to do this work, the evaluator must know the difference, which also depends on jurisdiction.
- Pedophiles—subtypes and morphologies—including the most likely culprits, how they operate, and "Stranger Danger."
- Internet Porn and the "victimless crime"—are they sex offenders, predators, or something else—and how does the use of internet porn predict offending against real live victims?
- Rapists and serial killers—can a serial killer not be a sexual predator?
- Rapist Morphology—because jargon is important.
- Sex offender risk assessment instruments—how they work

and how they do not—the theory behind using these instruments and how to use them with the appropriate caution.
- Sadists versus non-sadists—and what is sadism?
- Mentally ill and mentally retarded offenders—the "politically incorrect offender."

All topics—What is a sexual offense? In order to understand sex offenders their offenses must be identified—and will include case presentations and discussion. This workshop is intended to be an overview of all the types of sexual predators, how to identify them, how to perform risk assessments, and what clues to look for in their evaluations.

Sex Offenders, Rapists, Pedophiles

W10 Quality Assurance in Human Identification

John E. Byrd, PhD, 95-033 Hokuiwa St, No 51, Mililani, HI 96853-5530; and Thomas D. Holland, PhD*, Vincent J. Sava, MA*, Bradford Byrnes, LLM* and Stephanie R. Ah Sam, MS*, Hickam AFB, Central ID Lab, 310 Worchester Ave, HI 96853*

After attending this workshop, attendees will: (1) understand the basic quality assurance principles and measures applicable to human identification; (2) learn the unique challenges faced by professionals involved in human identification when striving to have their facilities, procedures, and casework meet the standards demanded by the criminal justice and medical-legal systems; and, (3) utilize the material presented to formulate a quality assurance program for their organization.

This presentation will impact the forensic science community by demonstrating how quality assurance in forensic laboratories and forensic programs has led to objective and measurable standards of performance that ultimately strengthen and elevate the forensic profession as a whole.

Quality Assurance Programs (QAP) and activities in forensic laboratories have been a growing trend over the past decade. The publication of the National Academy of Sciences Report: *Strengthening Forensic Science in the United States: A Path Forward* and its recommendations have made quality assurance programs and accreditation an increasing priority for forensic human identification laboratories. Since 1999, the Joint POW/MIA Accounting Command (JPAC), Central Identification Laboratory (CIL) has implemented a stringent QAP to ensure the scientific integrity of its casework. The CIL's QAP ultimately led to its accreditation by the American Society of Crime Laboratory Directors Laboratory Accreditation Board (ASCLD-LAB) in 2003—the first forensic skeletal identification laboratory so credentialed. In 2008, the CIL was re-accredited under the ASCLD-LAB *International Program* using ISO 17025 Criteria.

The goals and objectives of this workshop are to introduce the attendee to the CIL's QAP and to convey the lessons learned resulting from its implementation and growth. The workshop begins with an overview of the CIL, its mission, and QAP. A video of the CIL's mission and operations is presented. In the latter, the concept of the scientific integrity of the CIL is discussed followed by a summary of the "Surety" model of quality assurance.

The participants become familiar with each measure that comprises the surety model of quality assurance. The importance of integrating and synchronizing all of the surety measures discussed during the workshop is continually stressed.

Issues that are currently at the forefront of forensic quality management are presented. These include:
- Professional qualifications and development
- Ethics
- Research and development
- Validation of technical procedures
- Uncertainty of measure
- Data management and information release

Infrastructure and support considerations necessary for a successful QAP are also presented. Surety measures addressed include, but are not limited to:
- Desired qualities of a laboratory manual and other vital documentation and their control
- Adequacy and safety of laboratory facilities
- Purchasing and contracting for laboratory services and supplies
- Policies and procedures conducive to a positive work environment
- Customer service programs and complaint procedures
- Evidence management and security
- Training and professional development

Gathering and interpreting evidence discusses quality assurance measures directly related to field operations and trace evidence casework. These surety measures include:
- Peer review process
- Preparation of analytical notes, test reports and other case documentation
- Photography and other imaging
- Maintenance, calibration, and performance checking of equipment
- Taphonomic effects and evidence conservation considerations
- Writing and editing standard operating procedures (SOPs)

Quality assurance procedures and programs are ineffective unless they are monitored, enforced, and subjected to corrective action when non-compliances are discovered. Monitoring and corrective actions outline how these are accomplished in the CIL. Discussed are a myriad of surety measures including:
- Proficiency testing
- Review of court testimony
- Internal and external audits
- Annual reports to, and management reviews with, top management
- Corrective action policies and procedures regarding personnel, technical procedures, facilities, etc.

In closing, the workshop discusses problems that hindered, and the processes that led to, the accreditations of the CIL. Surety assistance programs offered by the CIL are discussed in the event an attendee's organization desires assistance with their QAP or accreditation efforts. Additionally, the contributions, to date, of the Scientific Working Group in Forensic Anthropology (SWGANTH) to the human identification profession are briefly discussed.

Quality Assurance, Human Identification, Forensic Anthropology

W11 Bones and Children: An Interdisciplinary Approach to Forensic Issues

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After attending this workshop, attendees will be able to: (1) understand strengths and weaknesses of the various disciplines (pathology, anthropology, pediatrics, and radiology) that can evaluate fractures in children; (2) describe medical conditions that can mimic fractures or predispose pediatric populations to fractures; (3) recognize that the collaboration of various medical and forensic specialists is the most productive way to find and evaluate fractures; (4) understand the strengths and limitations of various techniques for constructing the biological profile (age, sex, ancestry, and stature) of the dead, and how various factors impact these determinations; and, (5) understand the techniques used in evaluating pediatric remains that may be decomposed, unidentified, and/or skeletonized.

This workshop will impact the forensic community by assisting in the evaluation and interpretation of fractures in children. The finding of

fractures in children, particularly the very young, can have profound medicolegal implications if the fractures are suggestive of inflicted injury. The clinical, radiological, histological, and anthropological aspects of the fracture may provide useful information as to the mechanism and/or age of the injury. Correlation with published studies and the experience of the pediatric and radiological communities may prove vital in assessing whether a fracture might be accidental, or whether the child may have a medical condition predisposing to—or mimicking—fractures. By bringing a variety of medical and forensic specialists together to examine all aspects of fractures—including accidental fractures, conditions that might mimic fractures, and conditions that can predispose to fractures in the pediatric population—this workshop will help attendees increase knowledge of fracture evaluation within their own disciplines, and develop a deeper appreciation for the understanding of fractures that other medical and forensic specialists can bring to the table. This workshop also will impact the forensic science community by going beyond fractures to examine skeletal features of children and techniques which can be used that may prove useful in estimating or establishing age, verifying identity, estimating time since death, and confirming the presence of antemortem trauma.

The complex intersection of skeletal trauma analysis, bone pathology, clinical pediatrics, and radiology is centered on children, and often necessitates a multidisciplinary approach. The presence of unexplained fractures in living and deceased children—particularly infants and the very young—may have profound implications for how injuries are assessed and deaths investigated. Pediatricians, radiologists, pathologists, and anthropologists all have much to offer in the assessment of the pediatric skeleton, but may be unaware of other disciplines' skills that can be brought to bear on questions of considerable legal and forensic significance. Furthermore, some diseases may mimic fractures, or predispose to fractures, and these conditions must be recognized to ensure proper treatment and/or avoid miscarriages of justice. Just as the finding and interpreting of fractures in children is different than in adults, so too is the recovery and identification of pediatric remains. Thermal injury, decomposition, postmortem changes, and a variety of other factors present significant challenges in recovery and identification.

This workshop will utilize multiple medical and forensic disciplines (anthropology, pathology, radiology, and pediatrics) to examine features of children's bones that have forensic and legal implications: the recognition and interpretation of fractures; and, aspects of recovering, identifying, and evaluating skeletonized or decomposed pediatric remains. A significant portion of the workshop will be dedicated to explaining and demonstrating how various disciplines, working together, can provide more and better information and stronger conclusions than any one discipline alone. Specific examples of the multidisciplinary approach to the pediatric skeleton will include the complementary expertise of the pathologist and anthropologist in finding and interpreting fractures, the radiological and pathological correlation of fractures, clinical and radiological consultation with regard to pediatric development and likely fracture mechanisms, clinical and radiological features of diseases that mimic or predispose to fractures, and the multidisciplinary approach to the recovery and interpretation of pediatric skeletal remains. Historical and technical aspects of the various disciplines: anthropology (fracture identification, tissue maceration, fracture photography, assessment of healing, skeletal recovery, techniques for aging); pathology (fracture identification, specimen handling, bone development, normal and abnormal bone histology); radiology (the historical development of the recognition of abuse, techniques in radiological evaluation); and, pediatrics (examination of the patient, the differential diagnosis of skeletal findings) will be addressed.

Bones, Children, Fractures

W12 Principles and Applications of Liquid Chromatography Mass Spectrometry (LC/MS) for the Forensic Toxicologist

Peter R. Stout, PhD, and Megan Grabenauer, PhD*, RTI International, Center for Forensic Science, 3040 Cornwallis Rd, Research Triangle Park, NC 27709; and Michael Chen, PhD*, and Jeffrey P. Walterscheid, PhD*, Harris Co Inst of Forensic Sci, 1885 Old Spanish Tr, Houston, TX 77054*

After attending this presentation, attendees will understand the fundamentals of LC/MS for screening and confirmation of drugs and the advanced applications for forensic toxicology and insights into how results are reported. Attendees will benefit from and have the opportunity to discuss challenges at the end of the session.

This workshop will impact the forensic science community by raising awareness of the capabilities of LC/MS in detection strategies by accurate mass measurements and confirmation techniques by tandem mass spectrometry. This workshop will also explore cutting-edge analytical methods for evaluating routine and emerging drug compounds involved in impairment or death investigations.

The use of liquid chromatography time-of-flight mass spectrometry (LC/TOF-MS) for screening purposes has gained popularity in recent years. Different laboratories have developed screening methods for drugs of abuse, pesticides, veterinary drugs, doping agents, and other poisons in different matrices such as blood, urine, vitreous humor, hair, and even food. The original fame of accurate mass for confirming theoretical molecular formulas for synthetic compounds has grown to include its use in identification of unknowns and searching for target compounds in complex matrices. Mass measurements that are accurate to several decimal places coupled with creation of target drug compound list with retention time data allows for rapid, selective, and specific screening of target compounds.

The first session will address the basics of liquid chromatography based mass spectrometry. The commercialization of LC/MS permits the use of gentle electrospray ionization, while providing accurate mass and retention time data to identify analytes. Instrumental variants that use time-of-flight analysis provides an expansive technique for screening a large number of known and unknown analyte possibilities, while tandem-mass hardware is ideal for confirmation and quantitative analyses. A wide array of drugs, metabolites, adulterants, and other exotic compounds can be monitored by these techniques.

There are extensive classes of known synthetic cannabinoids and cathinone derivatives yet limited panels of available reference standards. These groups have quickly become an emerging threat to public safety due to their accessibility and lack of detection by routine screens. Furthermore, these drugs continue to evolve as manufacturers creatively modify side chains to complicate identification of new drugs and alter drug potency. The second presentation will address this problem by applying a technique of identifying compounds by accurate mass defect filtering. This approach allows the analyst to distinguish unique signals from matrix elements and other components that may obscure significant findings in unknown samples.

Once scans have revealed the presence of compounds of interest, it is necessary to authenticate the identity by establishing the concentration in comparison to a standard. However, there are many pitfalls that can obfuscate true proof of the analyte in question. Isobaric compounds are isomers that share the same molecular formula, mass, and possibly the same fragmentation ions or retention time. Techniques for extraction, chromatography, and mass spectrometry confirmations will be outlined. Choices of solvents, gradient profiles, specification of daughter ions, and, collision energies to enhance sensitivity and specificity in unambiguously defining the compound by liquid chromatography tandem mass spectrometry (LC/MS/MS) will be discussed.

From screening to confirmation, the data is assembled for reporting a result in the final analysis. The interpretation of data for toxicology reports requires knowledge of anticipated mass, retention time, and relative abundance of daughter ion ratios. Lastly, attendees will be

shown how to discern supportive data from suspect outcomes, and evaluate the veracity of the declared determination.

LC/MS/MS, LC/TOF-MS, Toxicology

W13 Calculating Likelihood Ratios Incorporating a Probability of Drop-Out: Introducing Lab Retriever — A Free and User-Friendly Software Program

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After attending this workshop, attendees will: (1) gain an understanding of how to solve challenges encountered by analysts in interpreting and weighting difficult DNA profiles; and, (2) learn the basics and mechanics of a free user-friendly software program to calculate statistics based on maximum profile information.

This presentation will impact the forensic science community by addressing one of the most difficult and prevalent challenges that forensic DNA laboratories face today—the interpretation and statistical weighting of complex DNA samples.

One of the most difficult and prevalent challenges that forensic DNA laboratories face today is the interpretation and statistical weighting of complex DNA samples. The forensic DNA community has struggled to provide reliable statistics for these ambiguous profiles. In a well-intentioned attempt to be "conservative," much of the information in the profile often is simply ignored. Far from being safe, this approach is dangerous in that it can lead to incorrect or grossly mis-weighted conclusions.

It is believed that the most reliable scientific interpretation and weighting of forensic DNA profiles should use as much information as possible. While general agreement exists that a likelihood ratio (LR) incorporating a probability of drop-out ($P(D_O)$) is the gold standard, implementation has been difficult for a number of reasons. First, although several automated tools to perform LRs in general have been proffered, they have been opaque, expensive, and not particularly user friendly. Second, in order to maximize the utility of LRs for challenging samples, it is necessary to incorporate a $P(D_O)$. Although a vast repository of data is available from the validation studies of forensic DNA laboratories, this information has not yet been mined for its value in empirically estimating drop-out probabilities. Third, it is critical to use a scientifically-determined analytical threshold to maximize the data used to generate a statistical weight.

The elements required to perform a LR with a $P(D_O)$, in particular the empirical determination of an analytical threshold and the empirical determination of the $P(D_O)$ will be discussed. A software tool that includes an easily-accessible user interface will be introduced. The Lab Retriever software, including the back-end computer code will be freely available without charge.

Approaches to assessing the weight of challenging samples — The advantages and limitations of several different approaches to assessing the weight of forensic DNA evidence will be discussed. These include the use of a stochastic threshold, the "2p rule," and a likelihood ratio (LR) that explicitly models the possibility of drop-out. Using current research results, Incorporation of drop-out leads to accurate estimates of the LR will be demonstrated. Similarly, data that the stochastic threshold approach often heavily underestimates the strength of the evidence and can lead to false exclusions will be presented. Further, results demonstrating that the "2p rule" performs similarly to the LR approach for some of the same situations will be shown.

Empirical determination of an analytical threshold — How thresholds currently employed by DNA labs are frequently too high and true DNA peaks left undetected, resulting in the abandonment of valuable information from difficult samples will be explained. We will show how an empirically derived analytical threshold assists in properly calculating an LR with a $P(D_O)$. How to determine an appropriate analytical threshold as the first step in preparing data for input into the "Lab Retriever" computer program will be demonstrated.

Empirical determination of $P(D_O)$ — A major impediment to implementing LR with $P(D_O)$ has been the lack of an empirically determined $P(D_O)$. How to determine an appropriate range of $P(D_O)$ for use with Lab Retriever, from data that every DNA laboratory has produced during validation will be demonstrated. The resulting algorithm is used to estimate the $P(D_O)$ for evidence samples.

Introduction to Lab Retriever — Lab Retriever, (Buckleton and Balding 2009, modified by Lohmueller) a program to calculate LRs with drop-out will be introduced. How data from an ambiguous profile is prepared for LR analysis will be demonstrated. Workshop attendees will participate in a practical exercise to reinforce their understanding of selection and preparation of data for use in Lab Retriever. Participants will be instructed on using the software, and will be provided with materials with which to practice their newly acquired knowledge and skills at home.

Low Template DNA, Likelihood Ratio, Dropout Probability

W14 *Melendez-Diaz*, *Bullcoming*, and *Williams*: Scientific Evidence and the Right to Confrontation

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After attending this workshop, attendees will be able to: (1) discuss the courts' interpretation of the Sixth Amendment on right to confrontation; (2) assess the impact of the rulings on forensic laboratory operations; and, (3) consider aspects of these rulings from perspectives other than their own.

This presentation will impact the forensic science community by educating forensic laboratory management and personnel about the requirements courts place on forensic science testimony, while educating attorneys about the impact of these rulings on laboratory operations and encouraging dialog between scientists and lawyers on meeting constitutional obligations while practically allocating resources within laboratories.

This workshop will examine the impact of three recent Supreme Court rulings on the right of defendants to confront expert witnesses at trial. The program will consider the issue from the perspectives of an academic constitutional law, prosecution, defense, and forensic laboratory director perspective, and will include an update on new appellate cases.

The Sixth Amendment to the United States Constitution guarantees that a defendant will have the right to confront their accuser. Becoming effective in 1791 as part of the Bill of Rights, this amendment was designed to help ensure fairness in criminal prosecutions and goes back to earlier European law. But in the age of electronic communications, video conferencing, automated testing, increasingly complex scientific evidence, and constrained forensic laboratory resources, how is that to be interpreted in 2012?

This workshop considers three important rulings issued by the U.S. Supreme Court in the last four years concerning the admissibility of laboratory results in criminal cases: *Melendez-Diaz vs. Massachusetts*, *Bullcoming vs. New Mexico*, and most recently *Williams vs. Illinois*.

These cases address, in part, the issue of what constitutes testimonial evidence, and who is permitted to testify to what aspects of scientific evidence under some case specific conditions. The Court, however, leaves many questions unanswered, including who is the "analyst" for purposes of confrontation, and can a scientist testify about their opinion on scientific evidence not admitted?

Courts interpretations of these cases has a significant impact on the presentation of scientific evidence in court, management of forensic laboratory resources, and the outcomes of trials in major crime cases.

This workshop will present four perspectives on the issue, beginning with a consideration of the language of the sixth amendment, its context in the time it was adopted, and subsequent interpretation in United States case law. This is followed by presentations between appellate attorneys on the state and defense side both with extensive experience in the presentation of scientific evidence. Perspectives on legal and practical aspects of litigating cases with forensic evidence will be presented and followed by the perspective of a forensic laboratory director responsible for planning laboratory operations, assigning job duties, and ensuring scientist availability for court in a laboratory environment where typically many people are involved in the analysis of a single piece of evidence.

The session concludes with a moderated discussion by the panel of presenters and a question and answer session with the participants.

***Melendez-Diaz*, Sixth Amendment, Testimony**

W15 Improving the Effectiveness of Forensic Service: Using the Foresight Project as a Platform for Quality

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After attending this workshop, attendees will be: (1) able to use basic metrics to evaluate the effectiveness and costs of their forensic services; and, (2) provided a platform for bench-marking with other forensic providers.

The workshop will impact the forensic science community by providing tools that will help improve operations of sections, units, or laboratories, while keeping in mind the importance of effectiveness and efficiency to ensure accuracy, quality, and timely provision of forensic services.

Forensic service providers are—in essence—non-profit, production-oriented organizations staffed largely by knowledge workers. Forensic scientists as knowledge workers take evidence and data and convert them into knowledge in the form of reports and testimony. They specialize in these transactions and, therefore, simplify them for the benefit of the criminal justice system; the investigators or attorneys do not need to find numerous individuals to conduct the specific examinations required for a case. As long as the costs of providing these services externally do not exceed the costs of their internal provision, for example, by a government forensic laboratory, then the organization can prosper. If the government laboratory costs are greater than the cost of finding private laboratories to provide services, then the organization may be re-evaluated. Comparatively, non-profit and for-profit organizations are similar in some ways (money is an input for both) yet different (money, in the form of profits, is an output only for the private sector). Non-profits must therefore measure success in other ways, such as "low cost" or "cost effective." Forensic service providers and their parent organizations use terms such as "cost-effective" vaguely without reference to other disciplines which use these as well-defined technical terms in evaluative phrases or formulae. Despite the great concern and administrative angst over forensic service providers' "performance" and "capacity," these metrics go undefined as industry standards.

Managers of scientific laboratories see themselves as scientists first and managers second; consequently, they tend to devalue the managerial aspects of their jobs. Despite—or perhaps because of—this lack of explicit understanding, forensic service providers are held accountable by one or more agencies for their performance based on non-standard, ill-defined, or non-existent criteria. Successes and improvements go unrecognized and opportunities for advancing the mission and goals of the organization are squandered. The stakes for forensic laboratories are high given the importance of quality science to the criminal justice system. The need for training and support in forensic laboratory management has been recognized for many years but little has been done to transition the tools of business to the forensic laboratory environment.

This workshop will provide an overview on how to use simple business metrics to evaluate your forensic services, improve effectiveness and quality, and provide the supporting information to request or justify additional resources. The workshop uses tools and data from the FORESIGHT Project, a business-guided self-evaluation of forensic science laboratories across North America. The process involves standardizing definitions for functional areas of the laboratory and metrics to evaluate work processes, linking financial information to work tasks and functions. Laboratory managers can then assess resource allocations, efficiencies, and value of services—the mission is to measure, preserve what works, and change what does not.

As an exercise to highlight the relevancy of the process, attendees are encouraged to develop the following data from their agencies for DNA, fingerprints, or toxicology (antemortem or postmortem): total financial expenditure in that area; number of full-time employees, including clerical; total compensation including benefits; number of cases submitted; and number of tests conducted. For this data, attendees are encouraged to use the standard definitions of the FORESIGHT Project as listed on the Laboratory Reporting and Analysis Tool (LabRAT).

Effectiveness, Efficiency, Foresight

W16 Forensic Entomological Aspects of Abuse and Neglect

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After attending this workshop, attendees will: (1) review the different scenarios in which insects are involved in abuse and neglect; (2) be shown how entomological investigations in such cases are carried out and how insect evidence can be of importance in the investigation; (3) be presented with the legal aspects of such cases; and, (4) be presented with criminal activity associated with using insects to make false accusations of abuse and neglect.

This presentation will impact the forensic science community by showing how abuse and neglect cases, in which insect evidence is present, are assessed and dealt with in the forensic investigation. Attendees will also become familiar with recognizing how insect evidence can be useful in addressing abuse and neglect cases in the criminal justice system.

Forensic entomology is often thought of as being involved with death investigation; however, insects can be of real concern in various aspects of abuse and neglect. Human insect infestations can sometimes become an issue with abuse and neglect of nursing home patients when bedding, clothing and hygiene of patients is at issue, with babies whose personal hygiene is not properly cared for, with the improper handling of wound cleansing, and various other situations in the medical profession. Some of the same insects (especially various fly species) associated with death investigations can also be involved in abuse and neglect cases. Many of these cases end up in the court system either as lawsuits or as criminal cases. This workshop will present the various situations in which insects can become involved in abuse and neglect with specific case studies presented. The criminal aspect of planting insects to appear as abuse and neglect will also be discussed.

Insect evidence can be found on contaminated clothing, bedding material, urine and fecal deposits, diapers, open sores and wounds, soiled wound dressings and bandages, among others. Entomological procedures will be presented to provide guidance in recognizing insect evidence associated with abuse and neglect along with collection and preservation techniques of insect evidence. Insects that are often seen in abuse and neglect cases are various species of flies, including blow flies, flesh flies, house flies, and others. Other insects and related arthropods that may be of issue include ants, beetles, bedbugs, mites and ticks, and spiders. Specific case studies will be presented to include abuse and neglect of elderly in care centers and nursing homes; infant abuse and neglect where insect invasion of clothing and feeding has occurred, and situations involving insect contaminated wounds from patients of outpatient caregivers. Also, cases of insect and related arthropod invasion and attack in which people claim to have been bitten will be presented. In addition to real cases of neglect and abuse, the criminal aspect of the planting of insects to appear as abuse and neglect and falsifying insect evidence will be discussed. Related to non-real cases is the area of delusory parasitosis, or entomophobia (a fear of insects). This will be presented to distinguish it from real cases of abuse and neglect. Discussion will be presented including the involvement roles of the entomologist, crime scene investigator, forensic nurse, and legal counsel.

The workshop will be concluded with discussion on the legal system's role in dealing with abuse and neglect cases from the aspect of lawsuits to criminal cases. How to determine the difference between lawsuits and criminal involvement will be discussed since this is often a point of contention. The role of the attorney and expert witness (entomologist, CSI, medical personnel, etc.) will be reviewed from both the plaintiff and defendant viewpoint.

Abuse and Neglect, Insect Evidence, Forensic Entomology

W17 Image Analysis — 3D Imaging and Virtopsies: Developments, Methods, and Reasoning About Images

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After attending this workshop, attendees will understand: (1) the issues with interpretation of images; (2) how virtopsies can help with interpretation; and, (3) what methods for validation exist.

This workshop will impact the forensic science community by: (1) explaining the different methods in imaging; (2) giving a relation between general image interpretation and interpretation of images in virtopsies and crime scene imaging; and, (3) briefing attendees on pitfalls, possibilities, and future expectations of image analysis.

The field of forensic imaging is expanding rapidly. There is a wide variation of images that can be used in forensic science, ranging from CT/MRI scans to photographs, video, three-dimensional acquisition of images with laser, or light to spectral and thermal imaging of crime scenes. The integration of these new technologies into working offices can be a challenge.

This workshop will discuss some of the issues related to this type of integration. Some of these technologies will be discussed and examples of how they are being used in offices today will be demonstrated. The use of 3D scanning provides better measurement and visualization as well as the ability to estimate error. The development of image databases allows exploitation of imagery over time and among different activities. Automated methods of image comparison and data mining allow exploitation of imagery that is transforming investigations. The use of computed tomography (CT) and magnetic resonance imaging (MRI) provides new and important information in the investigation of death, including better measurement of three-dimensional features and even, in the case of MR spectroscopy, biochemical analysis of tissues.

While these new technologies provide important benefits, they also present significant challenges. New technologies require interdisciplinary cooperation and training. Integrating these new technologies into existing organizational structures requires new infrastructure and different workflows. Rapid changes in large organizations can be a challenge, and those who are considering them should be aware of common organizational pitfalls. Practical issues of funding, organization, operation, and acceptance can be as or more important than the technical issues commonly considered.

Quality assurance is important to have in place when implementing these systems, especially in handling large amounts of data. For validation, a reference database of images can also be used to have a statistical approach on conclusions that can be drawn from these. Development of algorithms for comparison in databases and using these for computing likelihood ratios are presented, as well as a Bayesian framework that can be used for combining conclusions of different independent features. Examples are shown for related to morphometric comparison of 3D shapes in fields such as cartridge cases, shoeprints, and tool marks in databases. New developments in forensic science such as thermal imaging for determining the time of death at the crime scene and spectral imaging provide a new solution; however, it should be validated before being used in actual casework and/or integrated in the standard quality assurance system. Lessons of implementation of fast developing technology, such as digital evidence, can learn from the best practices from each other.

Further, modern requirements for justification of scientific conclusions require that one look at how these images are analyzed. The analysis of imagery includes determination of image authenticity, image integrity and provenance, and image content. This involves both technical issues and issues about how we think about images. It is important to examine how we look at images and what our reasoning processes are. The workshop will cover a broad range of imaging techniques, their validation and the implementation on conclusions that can be drawn from these from a broader perspective in a multidisciplinary approach.

Image Analysis, 3D Imaging, Virtopsies

W18 Staged Crime Scenes: Crime Scene Clues to Suspect Misdirection of the Investigation

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After attending this workshop, attendees will: (1) be able to identify the three new categories of staged scenes with case examples of different types of crimes where staging is prevalent; and, (2) learn the "red flags" or common findings in staged scenes.

This presentation will impact the forensic science community by introducing distinct categories of staging based on the motive of the offender's scene alteration. Ultimately, applying these categories may help in the quick recognition of scene alterations or staging.

In the course of their career, most detectives and forensic practitioners will come into contact with a staged crime scene; a scene that has been altered by the offender to either mislead a police investigation as to the true facts of the crime or for other reasons understood only by the offender. Staged scenes and "staging" are possible in nearly every type of criminal offense raging from property crimes such as arson and burglary to violent crimes such as homicide, child abuse, or sexual assaults.

To better understand the dynamics and the general nature of "staging" this workshop will introduce distinct categories of staged crime scenes based on the motive of the offender's scene alteration. The thrust of this workshop is to understand that the offender's actions to stage a

scene can actually be identified through common findings or "red flags" that are often found when scenes are altered. Further, that these same red flags may help in the quick recognition of scene alterations or staging, and based on new criteria may be divided into three separate and distinct categories. These categories are herein referred to as, Primary, Secondary, and Tertiary staging.

The Primary staged scene is intentionally altered or changed by the offender with criminal intent to misdirect a subsequent police investigation and can be further sub-categorized into two types: ad hoc and premeditated. The ad hoc subtype is staged without forethought and planning, at the spur of the moment after the event has taken place, and is generally intended to deflect attention away from the offender and true facts of the crime. The scene is considered premeditated when the offender pre-plans the scene alterations in accordance with a preconceived scenario. Premeditated staging is often designed to focus attention onto the staging and false evidence. Ultimately, through Primary staging the offender in effect creates a false reality that in his/her mind will successfully and with criminal intent, misdirect the police investigation.

Secondary staging involves the intentional alteration or manipulation of the crime scene or victim by an offender that is unrelated to misdirecting or diverting subsequent investigations. This is really a new category of staged scenes and would include such examples as posing the victim's body into sexual provocative positions as found in sexual homicides or other elements of what is more commonly known as "staging" such as covering the face or body in what is generally often described as depersonalization. The perpetrator's purpose in Secondary staging is not to misdirect the investigation, rather it is often something that is psychologically part and parcel to the crime, such as demeaning the victim or demonstrating ultimate control over the victim.

Tertiary staging defined consists of noncriminal, accidental, or innocent alterations; i.e.. changes to the original crime scene, generally by witnesses or family members, who find the victim and alter the scene without any criminal intent. An example would be a family member finding a loved one in an embarrassing position from an autoerotic misadventure and changing the scene to prevent embarrassment to the family. These types of alterations are best regarded as scene artifacts.

Primary Staging, Secondary Staging, Tertiary Staging

W19 Developments in Emerging and Designer Drug Markets 2013

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After attending this workshop, attendees will be able to: (1) evaluate their laboratory's preparedness for meeting the demands for designer drug testing; (2) describe the major features of the current illicit designer drug market; and, (3) implement additional resources for testing and interpretation.

This workshop will impact the forensic science community by providing updates on the most dynamic and rapidly changing area of forensic chemistry and toxicology in recent years, as well as providing forensic scientists with practical resources to implement at their own laboratories.

This workshop will provide updates on the rapidly changing field of designer drugs and their analogs. The workshop will present epidemiological information, trends reports from forensic laboratories, novel testing methodologies, and new analytical resources for criminalistics and toxicology laboratories.

Starting in 2009, a variety of products containing psychoactive drugs not previously known in the United States began to appear in the recreational drug marketplace. To date, these substances have been principally synthetic cannabinoid agonists or stimulant/hallucinogenic cathinone derivatives. The market is further changing; however, with Fatty Acid Amide Hydrolase (FAAH) Inhibitors, ketamine analogs, psychedelic phenethylamines, tryptamines, and benzofurans are now emerging in products and blends of multiple drugs. In the United States, federal authorities have moved to update scheduling and analog laws to keep up with the changes, but clandestine drug manufactures are constantly adding new classes.

This workshop begins with a series of presentations that evaluate the latest trends in the drug marketplace and help guide laboratories as to what drugs they should prioritize for method development and inclusion in their databases. These presentations include data from toxicological tests and street drug samples from labs throughout the United States as recorded in the U.S. NFLIS data set. The U.S. Drug Enforcement Administration (DEA) will also discuss their approach to categorization of the synthetic cannabinoid structural classes. Furthermore, the presentations include data from European laboratories where many of these drugs appear before reaching the United States' market. The epidemiological session will end with a discussion of the designer drug movement as a threat to the United States' domestic security.

The latter presentations will review developments in large scale screening for designer drugs as part of urine drug screening programs, and the challenges faced in matching screening technologies with confirmatory methods to make sure they remain compatible with each other and with the drug market. The session continues with presentations on elucidation of metabolic pathways for the synthetic cannabinoid and cathinone families of designer drugs, including use of *in vitro* and *in vivo* studies, and the application of GC/MS, LC/MS/MS and LC/TOF technologies. Publicly funded online databases and analytical data libraries will be described and approaches to their use in the identification of unknowns will be reviewed. The final sessions of this workshop will address the pharmacology and toxicology of methylenedioxypyrovalerone (MDPV), which emerged in mid-2012 as one of the most prevalent synthetic stimulant drugs, including animal behavioral studies which identify similarities between its effects and those of cocaine, explaining its popularity and potency. Also, discussed will be a collection of case reports of adverse events in users of synthetic drugs which have led to deaths and intoxications with fatal consequences.

Designer Drugs, Bath Salts, Cannabinoids

W20 Mobile Devices Examination

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After attending this workshop, attendees will be educated in the examination of mobile devices in a forensically sound manner through discussion of relevant topics:

Handling and Preservation of Mobile Devices – Attendees will understand proper procedures for collecting and handling mobile devices so as to preserve data for forensic examination.

Validation of Mobile Device Examination Methods – This discussion will present a proposed framework for validating mobile device examination methods and software tools in light of agency accreditation requirements. By learning to leverage available resources against their limitations, attendees will be able to relate the *Daubert* standards to the

challenges specific to codifying methodologies in an ever-evolving discipline.

Cell Phone and GPS Forensic Tool Classification System – Given the overwhelming prevalence that mobile devices have in our society, there is a need for a common framework to classify the plethora of tools released into the commercial marketplace. With the claims of software manufacturers often exaggerated, there is a specific need to understand not only how these tools work but when they should be used. Attendees will be provided with an overview of current commercial tools available for cell phone data extraction and will be able to effectively categorize any mobile device acquisition tool within a classification system.

GPS Forensics – Given the overwhelming prevalence that GPS devices have in our society there is a need to classify the plethora of tools released into the commercial marketplace. With the claims of software manufacturers often exaggerated, there is a specific need to comprehend in depth the way these GPS tools operate and the features they provide an analyst for data acquisition and analysis. Attendees will be provided with an overview of current commercial tools available for GPS data extraction/analysis and will be able to effectively categorize these tools within a classification system.

This presentation will impact the forensic science community by providing sound strategies and methodologies that can be directly applied to the examination of mobile devices and furthering the development of the digital forensic discipline.

Our world has become saturated with inexpensive digital devices. The ubiquitousness of these devices has changed the way we do everything, from staying in touch with friends on our "smartphone's" to finding the nearest gas station with our Global Positioning System (GPS). These devices are also frequently used by those engaged in criminal enterprise. Mobile devices themselves contain a wealth of information and intelligence for investigators; however the field of mobile digital device forensics is still in its infancy. In the forensic science community, mobile devices continue to be one of the most challenging areas for gaining and maintaining expertise. Mobile devices continue to evolve as new hardware/software combinations are updated several times a year. Trying to stay current with this evolving technology is a daunting task for any forensic analyst. This workshop will provide all knowledge levels the information to begin and/or maintain the ability to examine mobile devices. The process begins with the proper seizure of the device and how to ensure the device's data is maintained. Included will be a discussion of several different isolation methods that can be used to protect the mobile device from communicating while an examination is in progress. Isolation is necessary as any successful communication during the examination may result in a change in the data contained within the phone, or at worst, a complete forensic wipe of the device. There will be a discussion on the various isolation methods to protect the mobile device both on scene and in the laboratory. There will be a discussion on the need of verification to review the data located on the mobile device. This review correlates the information stored in the device with the data present in the data extraction report. The next step is the testing of the tools used for data extraction. Understanding what to expect from a given tool and then ensuring the information it provides matches what is stored in the mobile device is another critical step in the analysis process. An overview of validation as it pertains to the tools for mobile examination will be provided based upon ISO 17025.

A discussion of several of the commercially available GPS forensic tools will transpire. The workshop will conclude with a question and answer period. This workshop is designed to provide information and resources to computer forensic professionals regarding the examination of mobile devices from initial seizure to forensic analysis. Attendees will be provided with a rationale for the validation of mobile forensic examination methods. Additionally, detailed discussion will address classification of cell phone and GPS tools and extraction of deleted call history and text message records from the Apple® iPhone®.

Mobile Device, Computer Forensic, GPS

W21 Tracking John Wilkes Booth: An Interpretive Bus Tour

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After attending this workshop, attendees will understand the complexities in the tracking, the death, and the final burial of John Wilkes Booth, the assassin of President Lincoln, and the forensic science issues spawned by the assassination.

This presentation will impact the forensic science community by providing an interactive tour that will provide a fuller, real-world understanding of the escape route taken by John Wilkes Booth as well as the places and persons entwined with this place in American history.

This program will focus, through a moderated bus tour and site visitations, on the escape of John Wilkes Booth from Ford's Theatre to the homes of Mary Surratt and that of Dr. Samuel Mudd in Southern Maryland. Commentaries will be provided on the identification of the derringer as the murder weapon as well as the terminal medical care of Lincoln at the near-by Petersen House in Washington, DC and the identification of John Wilkes Booth.

The assassination of President Abraham Lincoln on Good Friday, April 14, 1865, and its aftermath put the spotlight on a number of historical matters of forensic significance. This one-day bus tour will highlight the places and events at the District of Columbia's Ford's Theatre, the site of the assassination, and at the Petersen House where Lincoln died and will follow the escape route of John Wilkes Booth to the Surratt Tavern and the home of Dr. Samuel A. Mudd in Southern Maryland where tours of the sites and discussions of the involvement of Mary Surratt and Dr. Mudd in the assassination conspiracy will be provided. Both Mary Surratt and Dr. Samuel A. Mudd were tried in a conspiracy trial by a military tribunal in Washington, DC with Mrs. Surratt being sentenced to be executed by hanging and Dr. Mudd being sentenced to a life term at Fort Jefferson, located in the Dry Tortugas ninety miles off the coast of Florida

During the tour, narrators will comment on various matters of historical and forensic significance in connection with the assassination of Abraham Lincoln including the unsuccessful medical attempt to save Lincoln's life and the killing, the identification, and the final burial of Booth at Baltimore's Green Mount Cemetery as well as the firearms identification of the gun used in the assassination that was left behind at Ford's Theater. Controversies are said to remain on a number of key matters in the history of the assassination, such as whether Booth was in fact killed at Garrett's Farm in Virginia and, if so, by whom among the tracking Union troops the deed was accomplished.

Assassination, John Wilkes Booth, Derringer

W22 Pediatric Pathology for Forensic Pathologists: What They Can Do for Us

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After attending this workshop, attendees will: (1) be able to recognize rare and fascinating pediatric natural diseases; (2) become familiar with consequences of common pediatric problems, such as prematurity, birth asphyxia, and birth trauma; (3) review placental pathology that is useful; (4) have a better understanding of the significance of a single abnormal finding in a forensic autopsy; (5) be able to recognize a number of natural diseases that can mimic neglect; and, (6) better understand when the situation calls for consulting a pediatric pathologist to assist in a forensic autopsy.

This presentation will impact the forensic science community by illustrating when consultation with a pediatric pathologist may be helpful to a forensic pathologist faced with a challenging case.

Pediatric cases sometimes present with baffling gross and microscopic findings, ranging from natural diseases which show up once in a forensic pathologist's lifetime, to complications of common diseases that are uncommon in forensic practice. For forensic pathologists, these baffling findings may obligate us to invest significant time searching references and discussing the case with colleagues, perhaps without finding the right diagnosis. If we know the diagnosis, we may not know the significance of the diagnosis to the cause and manner of death, or its consequences to the decedent's family. We may not know whether consultation with a pediatric pathologist will solve the problem, assist with part of the problem, or be of no use. Pediatric pathology may be a daunting field for forensic pathologists because of its encyclopedic knowledge base. To "prune the thicket" of pediatric pathology for the forensic pathologist, this workshop is presented in a case-based format utilizing actual forensic cases in which pediatric pathology resolved the mystery, or assisted us with understanding the significance of a natural finding, or its meaning for the family.

Progressing in sequence from the youngest possible patients, fetuses, with a look at placental pathology that is of use to the forensic pathologist as well as the consequences of birth trauma, the workshop examines cases from each stage of childhood, with attention to birth asphyxia and complications of prematurity. Unsuspected tumors, infections, metabolic disorders, congenital malformations and syndromes, and a number of rare disorders which may be genetic and significant for the family, along with predisposing factors of family concern, will be covered as we go on to discuss deaths in younger and older children, and finally, deaths from unexpected natural disease in teenagers.

Inflicted child abuse will not be the topic under discussion in this workshop; pediatric pathology will take center stage. Abusive head injury, unsafe sleeping deaths, and other primary forensic topics, will not be presented; however, when there is a question of fatal neglect, pediatric pathology may offer invaluable assistance by identifying alternate natural explanations for death. Natural conditions that mimic neglect in infants and children will be a topic of special attention.

The workshop will also delve into what we might do after identifying a finding of uncertain significance. These cases will be presented under the rubric of "All I have is this: does it matter?" Along with these cases, to hone their decision-making skills, participants will be involved in self-directed learning activities. Case histories with gross and microscopic findings will be made available, to allow the participants to diagnose and judge for themselves whether pediatric pathology consultation would be of some help, great help, or no help at all.

By the end, participants will learn a case-based format presenting a range of common and uncommon problems in pediatric pathology as they present forensically. Detection of rare diseases has always formed part of the reward of practicing pediatric pathology, and the forensic pathologist may be in the best position to identify these diseases at autopsy and to share them with our pediatric pathology colleagues, who can help us in turn to help the family. Participants will better understand when forensic pathology and the families we serve can benefit from pediatric pathology, and perhaps, vice versa.

Pediatric, Forensic, Pathology

W23 DNA in Real Time: Amplifying Productivity in Today's Forensic Laboratory

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After attending this workshop, attendees will gain the tools to be able: (1) to recognize opportunities for change within their own laboratories; and, (2) to prepare attendees to develop and implement their own strategies for improving DNA testing services.

This workshop will impact the forensic science community by providing a laboratory with information that enables it to recognize the potential for change within its casework operations, to define a roadmap for improvement, to recognize factors that mitigate the risks of change, and to achieve greater productivity and efficiency while maintaining quality.

Using the experiences of a forensic DNA laboratory, the goal of this workshop is to provide information that enables a DNA laboratory to recognize the potential for change within its casework operations, define a roadmap for improvement, recognize factors that mitigate the risks of change, and achieve greater productivity and efficiency while maintaining quality.

Today's forensic DNA testing laboratory is faced with unprecedented demands from the criminal justice and intelligence communities, and many laboratories are challenged to meet those needs given available resources. Failure to keep pace with case submissions results in a need to prioritize cases; which effectively decreases the utility of CODIS for cases with investigative needs such as missing persons and no-suspect cases. Furthermore, backlogs can drive the creation of case acceptance policies that limit testing for multiple-item submissions and thereby minimize the utility of DNA testing for law enforcement.

This workshop is not just about big laboratories doing big things but rather it stresses universal approaches to maximize production in DNA laboratories of any size. Topics of discussion will help a laboratory to evaluate and establish an infrastructure to eliminate existing case backlogs, to achieve targeted service times, to increase technical capacity and to improve typing success rates. In the long term, the strategies conveyed can prepare the laboratory for unanticipated trends in case submission and increased service requirements, and can enable the laboratory to implement new testing services and continually evolve with technology.

Using the re-engineered nuclear DNA laboratory at the FBI as a model, presentations will detail changes implemented from 2010 to the present, including: (1) progressive strategies to expand and enhance DNA testing services; (2) research and validation studies; (3) integration of information technology to manage and automate scientific processes and robotics; (4) workflow strategies; (5) workforce repurposing in response to changing needs; and, (6) maintenance of the quality system in the new laboratory environment. These changes have enabled the FBI to achieve an unprecedented level of casework productivity, including elimination of a backlog of some 2,700 cases and the typing of nearly 13,000 samples in 2011; to improve turnaround time from more than 600 days to 30 days on average; to increase the typing success in missing persons cases from approximately 10 to 80 percent, and to contribute more than 3,400 haplotypes to the US Y-STR Database.

Forensic DNA, Automation, STR Analysis

A1 Detecting Organic Gunshot Residue by Electrospray Ionization: Ion Mobility Spectrometry

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The goal of this presentation is to raise awareness of the need to improve the current method of analyzing gunshot residue and learn of a novel technique that is promising to this application.

This research will impact the forensic science community by presenting a novel, fast, and reliable detection for gunshot residue by a swab of the hand of a suspected shooter.

A method was developed to characterize organic components of Gunshot Residue (GSR) using electrospray Ionization-Ion Mobility Spectrometry (ESI-IMS) as well as ^{63}Ni-ion mobility spectrometry. As a field test, IMS displays potential for providing probabilistic determinations of shooter vs. non-shooter based on statistical pattern matching of the combination of compounds common to GSR. There is a need for reliable, rapid field screening of hands because current gunshot residue analysis techniques are merely presumptive. Due to many factors such as high false positive rates and low informational content, traditional color-based presumptive testing is increasingly used for visualizing reagents for distance determinations. The Griess reagent, a color-based GSR test, detects the presence of nitrites, which are heavily and naturally present in the environment. Organic Gunshot Residue (OGSR) analysis may provide valuable information when inorganic gunshot residue cannot be detected, or there is a question as to whether the metals found on a hand are distinctive compounds of firing a handgun. The combination of organic and inorganic compounds unique to GSR increases the confidence of a shooter/non-shooter decision. OGSR come from energetics and propellant additives such as stabilizers, plasticizers, flash inhibitors, coolants, moderants, surface lubricants, and anti-wear additives. Recent analytical studies of OGSR have targeted diphenylamine, nitrocellulose, nitroglycerin, and 2,4-dinitrotoluene; this work has demonstrated that phthalates and nitroso-derivatives of diphenylamine are also detectable post-firing using ion mobility spectrometry. This study is unique in that the target compound list has been extended to include organic combustion by-products that are specific to firing a handgun. The combination of these unique compounds should decrease the rate of false positives that are detected on the hand of a suspected shooter. Additionally, detection of a combination of propellant ingredients and combustion products significantly increases the confidence of a field shooter/non-shooter decision. The combustion by-product compounds were identified after collection of the vapors associated with small arms discharge using an enclosed firing box. The analysis of the collected vapors was performed on ESI-IMS and confirmed using gas chromatography-mass spectrometry and standards when available. These standards were then analyzed using ^{63}Ni and ESI-IMS to determine figures of merit for the analytical methods. Additional experiments were undertaken with hand swabs taken from individuals after discharging a gun (as well as individuals who did not discharge a gun) and it was determined that combustion products are detectable from the hands of shooters using IMS. The results were also confirmed with ESI-IMS-MS. Development of swabbing and extraction parameters will be discussed. Mobility spectra collected from hand swabs were incorporated into multivariate statistical models that ultimately provide the foundation for probabilistic assessment of shooter/non-shooter. These results will be presented and discussed.

Gunshot Residue, ESI, IMS

A2 Kinetics of Ion Mobility Spectrometer Sampling With Gunpowder and Methamphetamine

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After attending this presentation, attendees will understand that, across multiple forms of IMS sampling, whether positive or negative, results in an adjustment of the area of a spike following a set pattern of kinetics.

This presentation will impact the forensic science community by presenting groundwork, such as on a spectrum when it no longer becomes possible to extract the sample from the IMS swab and identify it in a GC/MS.

Ion Mobility Spectroscopy (IMS) is a commonly used instrument for field detection of drugs and explosives. A major disadvantage of IMS testing is its need to vaporize and therefore destroy part of the sample applied to the swab, limiting repetitive testing that may be required in a criminal case by both the defense and the prosecution. This research was undertaken to study the diminution of sample over repeated IMS samplings. A search of the literature revealed no previous studies of this kind.

The testing regime examined detection after continuous repetition using diphenylamine, methamphetamine, GSS, nitroglycerin, and ephedrine, starting with 1µl amounts to minimize the sample needed, and also to prevent overloading the device. A swab was inserted into the machine repeatedly with a spectrum taken of each run, until the sample could no longer be detected by the IMS due to the sample's peak blending into the small amount of background noise. The chemicals varied in length of retention from as short as three runs, to as long as 89 consecutive runs with clean cycles every 20 runs to assure that the signal being observed was not the result of carry-over. After data collection was complete, a Gaussian fit was taken of the average peak spectrum and cataloged in an excel spreadsheet, wherein it was found that every chemical tested seemed to follow a similar pattern. All chemicals decayed rapidly at first being diminished by half within the first two runs and then quickly declining until a nearly flat line is achieved. Upon further analysis, a distinct pattern was observed when plotting the natural log (ln) and inverse area of the peaks. The plot revealed a pattern pertaining to second order kinetics. This was confirmed by applying the form $1/kt+1/[A_0]$ to all peaks and running the numbers through the formula, with the same areas being concluded once k and A_0 were computed.

This study indicates that in both negative and positive mode of the particle analysis sampling in an IMS instrument, the major components of both gunpowder and methamphetamine follow a pattern of second order kinetics when repeatedly submitted to the spectrometer. It is hoped that continued research will determine at which point samples tested in the field are still suitable for subsequent testing in the laboratory by GC/MS.

Kinetics, IMS, Analysis

A3 Gunshot Residue Signal Decay and Back-Extraction Analysis Using Thermal Desorption Ion Mobility Spectrometry

Marriah C. Ellington, BS, 235 Dewey St, Morgantown, WV 26501; Joshuah M. Usilton, BS*, 1331 Rising Sun Rd, Camden, DE 19934; and Suzanne Bell, PhD*, West Virginia Univ, 1600 University Ave, Oglebay Hall, Rm 208, Morgantown, WV 26506-6121*

After attending this presentation, attendees will gain perception of the capabilities and functionality of the thermal desorption ion mobility spectrometer, focusing to illuminate the ability of the Thermal Desorption Ion Mobility Spectrometry (TD-IMS) to analyze, qualify, and quantify organic gunshot residue.

This presentation will impact the forensic science community by supplying a sturdy and portable preliminary testing device to analysts in the field, thus limiting the possibility of contaminating evidence en route to the lab. Evidence used in the TD-IMS can be recycled as well, allowing more to be done with less.

Current popular methods of gunshot residue analysis include Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS). SEM characterizes the morphology of particulates to determine if they are spherical, a sign that vaporized metallic Gunshot Residue (GSR) condensed upon cooling. EDS identifies whether these spheres consist of barium, antimony, and lead, the three most common metals in GSR. However, with lead-free bullets on the market and odd morphologies of particles possible, other methods of analysis would prove beneficial.

TD-IMS is primarily used as a field device for detecting narcotics and explosives. It has great potential for analysis of Organic Gunshot Residue (OGSR), an often-overlooked source of evidence from propellant combustion. The focus of this project was to develop a hand-swabbing method in which one swab could be interrogated using TD-IMS, back-extracted, then analyzed using Gas Chromatography-Mass Spectrometry (GC/MS). The ability to successfully extract after a thermal desorption cycle depends on the amount of organic material collected on the swab, the contact area of the desorber with the swab, and the efficiency of the thermal desorption process.

First, each thermal desorption cycle loss was evaluated using spiked swabs. Several different target analytes were studied including nitroglycerin and diphenylamine, both single-based gunshot surveillance standards. For each analysis, a swab was spiked with a known amount of the target analyte at the parts-per-million level in solvent. The swabs were allowed to dry and then repeatedly subjected to TD-IMS. In all cases, the decay of the peak of interest followed a second-order kinetics model. This suggests that the swabs could be analyzed first by TD-IMS and then extracted for further instrumental confirmation.

GSR field samples from a firing range were similarly analyzed and distinctive TD-IMS patterns were observed.

An isopropanol wiping method was developed to collect hand samples from a random population that also purposely included those who handle weapons and ammunition such as gun shop owners and police officers. This database was used to establish a range of typical background levels of compounds of interest and to develop TD-IMS signal-to-noise ratios. Results to date will be discussed, as will results from authentic post-firing samples.

This research displays the potential of TD-IMS as a screening method for detection of organic gunshot residue. A single swab collected post-firing can be used for both screening and confirmation, a luxury not afforded by older color testing methods and current SEM/EDS sample collection procedures. The work also further validates the potential of organic components of GSR as viable analytes in forensic GSR testing.

Gunshot Residue, TD-IMS, Field Instrument

A4 Substrate Interference and the Spectroscopic Identification of Body Fluids

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After attending this presentation, attendees will better understand recent methodological advancements toward the non-destructive, on-field detection of body fluids using Raman spectroscopy. Specifically, the issue of substrate interference will be addressed. Attendees learn the research goal and will be presented with the data treatment and instrumental factors, which would be a benefit in this regard. This work has specific implications toward the development of a specialized, automatic instrument, which could be developed to identify body fluids at a crime scene.

This presentation will impact the forensic science community by noting the presence of Raman spectroscopy to have ample applications to forensic sciences, especially in the area of crime scene investigation.

Raman spectroscopy has been found to have ample applications to forensic sciences, especially in the area of crime scene investigation. The technique is based on the detection of scattered light by a sample upon irradiation by a laser light source. This approach is typically rapid, non-destructive, field applicable and confirmatory in the identification of unknowns. The identification of body fluids using this technique would represent a significant improvement over the current methodology, which encompasses a range of destructive chemical assays that provide only presumptive identification. However, the issue of substrate interference is a major hurdle inherent in this approach. With Raman spectroscopy, body fluids exhibit a weak-to-moderate signal while the signal from the material directly underneath can be quite strong. Eliminating the substrates' interference is a prerequisite to confident identification.

Although substrate interference is studied here in the scope of body fluid identification, it is an ubiquitous issue in rapid spectroscopic analysis methods. Until now, the solution to the problem of substrate interference has been to collect the sample for later analysis on a preferential, non-interfering surface. However, the requirements of the forensic community considerably favor analysis *in situ*.

Using blood and semen as model fluids, substrate interference is explored using common materials expected to be present at a crime scene (fabrics, glass, tile, skin, etc.). A Renishaw Raman microscope with variable laser excitation was used in conjunction with a motorized automatic stage. Matlab and WiRE software was used for data interpretation and analysis. A variety of experimental parameters were evaluated with simulated evidence to identify the best conditions to simplify identification. Such parameters evaluated were objective strength, laser excitation, simple spectral subtraction, advanced automatic subtraction, and statistical modeling.

The fluid in question and the nature of the substrate were found to be determinative of the most favorable experimental conditions. In the simplest example, the interference from glass beneath a bloodstain is negligible with careful selection of laser excitation. An example of a complex scenario would be detecting semen on a blended dyed fabric. For this type of evidence, statistical modeling was required to find areas of high fluid concentration before spectral subtraction. It was also discovered that blood could be easily identified from a cotton collection swab after mild data treatment. Identification is possible using auto correlation and previously developed Raman spectroscopic signatures, which provide a statistical measure of certainty.

Substrate interference is one of the final analytical obstacles impeding real-world Raman spectroscopic identification of body fluids. This work demonstrates that a non-specialized common Raman instrument and modest data treatment are capable of overcoming this challenge.

Spectroscopy, Serology, Biological Evidence

A5 Comparison of Six Latent Bloodstain Reagents for Sensitivity, Specificity, and Impact on DNA Yield and Quality

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After attending this presentation, attendees will benefit from scientific observations on a variety of latent bloodstainenhancing reagents based on their sensitivity, specificity, longevity of reaction, ease of use, as well as impact on downstream DNA analysis.

This presentation will impact the forensic science community by providing aid in selecting latent bloodstaintesting methods that allows for high-quality results, while also aiding in efficiency of the laboratory. Because backlogs are a constant companion to the forensic science laboratory, efficiency is essential in proper casework management.

Due to the ease of use as well as the enhancements made by the manufacturers which allows for greater longevity in the reaction, the manufactured reagents will provide a suitable alternative to the traditional recipes many laboratories currently prepare in-house.

While blood is typically the most visually apparent physiological fluid present at a crime scene, or on items of evidence in a forensic laboratory, there are situations in which the blood is latent, or not visible to the naked eye. In these cases, some form of enhancement is required for the visualization and eventual collection of the bloodstains. The latency of the blood is often due to efforts to clean or remove the blood. As a result, any latent blood present at a crime scene has been thoroughly diluted. Additionally, there are often chemicals present in the area that is being tested that can potentially stymie future serological, chemical, or DNA testing. A latent blood test to aid the forensic scientist in locating the bloodstains must be sensitive, so that it can detect these low levels of blood, as well as specific, so that it properly identifies blood and not one of the many potential cleaning agents or other chemical and natural insults that could cause false positive or false negative results. A pitfall of latent bloodstain tests is the ephemeral quality of the reaction; the optimum reagent would present a reaction that could be easily viewed and potentially photographed without requiring further applications of reagent. Finally, the ideal reagent should not impact the downstream DNA testing that is required for identification of the source of the blood.

A wide variety of manufactured reagents, as well formulas for preparation of in-house reagents, are available to the forensic scientists. Six latent bloodstain reagents were selected for comparison. The reagents (luminol, fluorescein, fluorescein with thickener, Hemascein®, Tink's Starlight Bloodhound™, and BlueStar®) were applied to a variety of blood dilutions, substrates, and blood mixed with chemical and physiological fluid insults. The results of the testing, as well as observations on longevity of the results and ease of preparation, were noted. A selection of the blood dilutions was sampled for DNA analysis, to determine the impact on DNA yield and DNA quality. The selected samples were extracted on a variety of platforms, to monitor any variability that might result between an organic extraction method versus automated, silica-based extraction methods. Additionally, the impact on yield was measured through the quantitation of the DNA extractions. Finally, the quality of the DNA obtained from the tested samples was determined through the amplification and subsequent genetic analysis via capillary electrophoresis.

The manufactured reagents were observed to exhibit qualities commensurate or exceeding those of the in-house formulas. As a result, the manufactured reagents, while sometimes more expensive, provide an ease of use and quality of results that makes them a suitable, if not superior, alternative to the in-house reagents.

Latent Blood, Luminol, DNA

A6 Reliability and Reproducibility of Friction Ridge Edgeoscopy

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After attending this presentation, attendees will understand the problems involved in comparing exemplar prints to friction ridge prints recovered from crime scenes using edgeoscopy.

This presentation will impact the forensic science community by investigating the factors that affect ridge detail when fingerprints are deposited at crime scenes as compared to exemplar prints collected by the authorities.

In this study, the factors that affect ridge detail when fingerprints are deposited at crime scenes, and during the taking of exemplar prints, will be examined. These factors are: substrate, matrix, and pressurerelated deformations along with development media and anatomicalrelated distortions. Four of the most common methods of print development were used and evaluated for the level of detail in the recovered latent prints. These included: fingerprint powder, cyanoacrylate and iodine fuming, and development with ninhydrin. Each type of substrate, pressure, and development media combination will produce its own unique blend of distortions and deformations. Specific areas for edgeoscopy were selected and the exemplar prints were then compared to the developed latent prints to determine if these features could be distinguished and compared between both prints.

This study examined whether or not thirdlevel detail, namely edgeoscopy, could be distinguished among all of the distortions found in exemplars and latent prints. The first hypothesis tested was that variation in pressure and direction used to deposit fingerprints will create unpredictable deformation in edgeoscopy, even in exemplar prints. To do this, exemplars were deposited in five different directions onto photo paper to reduce ink bleed: roll to left, roll to right, roll down, roll up, and pressed vertically onto the paper. The second hypothesis tested whether prints lifted from surfaces typically found at crime scenes would produce the same level of detail as exemplars taken under ideal situations. Six edgeoscopy features were chosen at random for comparison across all latent and exemplar prints examined for hypotheses one and two. Analysis of these exemplar sets revealed that edgeoscopy features showed significant and inconsistent variation. Some features remained recognizable while others were distorted or obscured completely. The same areas of edgeoscopy were then evaluated on latent prints, deposited on a variety of surfaces and then developed using fingerprint powder, cyanoacrylate and iodine fuming, and ninhydrin. Some samples produced areas with excellent edgeoscopy reproduction. In the majority of the developed latent prints, however, the edgeoscopy details were unrecognizable. Ninhydrin in particular produced no observable edgeoscopy detail due to the fragmented appearance of the developed print.

Friction ridge prints play a vital part in criminal investigations. The public believes that latent prints are more reliable than they actually are in case investigations because of the CSI Effect. This study showed that edgeoscopy reproducibility is unpredictable. However, edgeoscopy could provide added supporting evidence in the rare occasions where this detail is recovered. This area of friction ridge print science warrants more research and evaluation. Methods for collection of exemplar prints that produce the highest level, and most reproducible amount, of third level detail have also yet to be determined. It is extremely important that everything possible be done to minimize distortion while taking exemplar prints. Without the detail present in an exemplar print to compare to, the detail recovered from a latent print will be useless to the investigator. Parallel research must also be done to determine the best methods for recovery of quality latent print ridge details. This is a large area of research due to the number of substrate and matrix combinations possible. Each will have a development method that is the most effective.

Friction Ridges, Latent Prints, Edgeoscopy

A7 Development of a Method for the Trace Analysis of Hexamethylenetriperoxide Diamine (HMTD) Using Liquid Chromatography/Atmospheric Pressure Chemical Ionization-Mass Spectrometry

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After attending this presentation, attendees will have a greater understanding for the prevalence of Hexamethylenetriperoxide Diamine (HMTD) in Improvised Explosive Devices (IEDs) and how to qualitatively detect trace/residue amounts of this peroxide explosive.

This presentation will impact the forensic science community by providing insight into the use Of Liquid Chromatography/Atmospheric Pressure Chemical Ionization-Mass Spectrometry (LC/APCI/MS) to analyze evidence related to IEDs suspected of containing HMTD.

Terrrorist groups are increasingly using peroxide explosives in IEDs. HMTD and Triactetone Triperoxide (TATP) are the most commonly used peroxide explosives and have similar explosive power to commercial and military high explosives. These compounds can be used as a main charge or an initiating explosive because they are extremely unstable, very sensitive to heat, friction, shock, and impact. Additionally, HMTD can be made from easily obtained retail materials, which results in it being a frequently identified component of IEDs. Consequently, with the increasing use of HMTD in terrorist explosive devices, it is imperative to be able to identify trace amounts of HMTD on items of evidence.

A qualitative method has been developed using LC/MS with an APCI ionization source in the full scan positive mode. Items suspected of containing trace amounts of HMTD are first extracted with deionized water for five minutes before filtering with 0.2µm syringe filters. Extracts are then injected into a separations module with a C18 column (150 x 2.1mm, 5µm particle size) and a solvent gradient of water: methanol, both containing 1.25mM ammonium nitrate. Lastly, an ion trap mass spectrometer is used for HMTD detection and identification.

Using the above method, HMTD elutes at approximately five minutes during an overall run time of 20 minutes. The observed mass spectrum contains m/z 207 and 224 that correspond to [HMTD-1]+ and [HMTD+NH4]+, respectively, in addition to 145, 177, and 209. Interference studies were performed by extracting different substrates to determine if anything present in commonly used materials will interfere with the assay. Results have shown that the following materials do not interfere with HMTD: cotton swabs, cotton balls, plastic scoops, wooden tongue depressors, rocky soil, and sandy soil. In addition, chemicals commonly used in HMTD synthesis were tested for interferences, and the following did not interfere with HMTD: hydrogen peroxide, reagent grade hexamine, reagent grade citric acid, sports tablet hexamine, and Gefen Sour Salt citric acid. TATP and HMTD were analyzed simultaneously, and results indicate that TATP is not an interference for the method.

An ion suppression/enhancement test was performed using a series of ten cotton swabs by fortifying the negative matrix extracts with HMTD before analysis, and the signal from the matrix samples was compared to the HMTD signal from a reference material at this same concentration. Also, matrix samples were processed and fortified with an HMTD standard and were analyzed every six hours following preparation for 72 hours to verify the processed sample stability. Lastly, the matrix limit of detection was determined by analyzing triplicates of matrix-matched reference materials fortified with HMTD reference material at 5, 10, and 15ppm levels.

This detection method yields sufficient peak resolution for the tested substrates and can qualitatively detect and identify HMTD at or above 10ppm.

Peroxide Explosives, HMTD, LC/MS

A8 Micro-FTIR-ATR Analysis and Statistical Validation of Dissimilar Inks on Paper

Gary H. Naisbitt, PhD, Andy V. Pham, and Bruce A. Jacoby, Utah Valley Univ, Criminal Justice Dept, MS 286, 800 W University Pkwy, Orem, UT 84058*

WITHDRAWN

A9 Statistical Discrimination of Explosive Precursors Using Data Gathered From High Resolution Fourier Transform Infrared Spectroscopy

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The goal of this presentation is to give attendees an understanding of the statistical analysis used to discriminate between potential explosive precursor materials based upon spectra produced using high-resolution Fourier Transform Infrared (FTIR) spectroscopy.

This presentation will impact the forensic science community by demonstrating the use of readily available statistical software to discriminate between similar infrared spectra, initially applied to the analysis of explosive precursors, but with the potential for use in other areas of forensic analysis.

The characterization and discrimination of different explosive precursors is an integral part of the information gathering used in the development of explosives detection security systems. Detection of precursors facilitates the interception of such materials prior to the completion of an explosive device. The ability to discriminate between explosive precursors of different origins facilitates accurate detection, providing greater strength of evidence against suspected bomb-makers.

The increased use of the so-called “homemade explosives” has resulted in a range of potential explosive precursors. As the name suggests, these explosive materials can be produced without a specialist laboratory from precursor materials that are easily purchased in small amounts. However, the precursor materials are often not in a pure form depending on their commercial purpose. For example, acetone is the active ingredient in many nail polish removers and it is combined with emollients, conditioners, perfumes, and colorants to enhance the product. Different brands with different ingredients have the potential to produce different infrared spectra, thereby giving the potential for discrimination. Depending upon the concentration of the primary ingredient, its spectrum may be masked by the other ingredients. In addition, many explosive precursor materials in their “off the shelf” form contain a relatively large amount of water, which also affects the infrared spectrum. Through previous work, highresolution FTIR spectroscopy has been demonstrated to be suitable for the characterization of laboratorygrade explosive materials and their precursors, as the technique produces information-rich spectra. The production of information-rich spectra helps to overcome the issues of masking and water interference. Discrimination may be possible from visually examining spectra of different precursor brands; however, statistical techniques can be used to detect minute differences between spectra which on the surface appear visually very similar. Moreover even in cases where the spectra appear visually different, statistical analysis can aid the discrimination of different “off the shelf” brands.

Three main statistical techniques were used to explore the best data mapping system for the complex data emerging from the analysis: cluster analysis, principal component analysis, and, Pearson correlation. The data presented here detail the development of a protocol for the stepwise application of data processing, followed by statistical analysis

for the discrimination of explosive precursors and the initial results obtained from the application of the protocol.

Statistics, Cluster Analysis, Explosives

A10 Explosives Analysis Using Gas Chromatography/Mass Spectrometry

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After attending this presentation, attendees will be able to assess their current laboratory techniques for analysis of problematic compounds and determine if the GC/MS method will improve trace explosives identification for a wider variety of compounds in relation to their current methods.

This presentation will benefit the forensic science community by showing laboratory personnel how to adjust their GC/MS equipment for the clearest mass spectra of high explosive compounds and their isomers.

This research will demonstrate those highexplosive compounds that do not survive typical GC/MS conditions. It also presents a new database of compounds for GC/MS, including common high explosives and related compounds. Such a complete database of these compounds is difficult to find, making this a useful tool for any laboratory.

More laboratories use GC/MS than any other technology for analytical purposes. Gas Chromatography (GC) is advantageous in trace analysis as it has a high resolving power, resulting in more efficient separations, which lead to better identifications. Mass Spectrometry (MS) is an accurate and reliable detector with the capability of examining a broad range of compounds and identifying them with specificity. The combination of GC with MS allows for improved separation of complex matrices and specific identification of trace analytes.

The greatest obstacle when analyzing highexplosive compounds by GC/MS is the high reactivity and propensity of the compounds to decompose rapidly at high temperatures. At high temperatures, the explosive compounds decompose and their mass spectra contain ions that can be found in a wide variety of molecular decompositions, making it difficult to produce and identify a unique explosive compound. In addition to the temperature challenges, active sites can be found throughout the chromatographic system attracting these explosive compounds and binding with them. The explosive compounds become lost in the system and never appear in the MS source to produce a spectrum.

This research includes method development for the following compounds: Trinitrotoluene (TNT), Cyclotrimethylenetrinitramine (RDX), Cyclotetramethylene-Tetranitramine (HMX), Pentaerythritol Tetranitrate (PETN), Dinitrotoluene (DNT), and Trinitrophenylmethylnitramine (tetryl). Optimum inlet temperatures for each of these compounds were determined in an attempt to decrease the effect of the most common decomposition location.

Three main inlet temperatures, 250˚C, 150˚C, and 80˚C, were tested with TNT, RDX, HMX, PETN, and Tetryl. Of these inlet temperatures, 250˚C was determined to be optimum for PETN, 150˚C for tetryl and RDX, and 80˚C for HMX. TNT was not affected by differing inlet temperatures. Furthermore, tetryl's mass spectrum matched N-methyl picramide, a product of tetryl hydrolysis, which was expected from reports in the literature. RDX showed breakdown resulting in a lower level of m/z 76 present in the spectrum. Similar data was collected from a total of 35 compounds including high explosives, their isomers, plasticizers, and others found in the presence of explosives.

This research will benefit the larger community of law enforcement, military, and even industry by providing more options to laboratories for explosive analysis. Forensic explosive analysis can identify compounds in improvised explosive devices (IEDs) and land mines in war zones to properly identify explosive components in pre- and postdetonation situations. Explosive manufacturers and research facilities would benefit from improved analytical techniques to determine the quality of their product and to evaluate changes that occur to bulk materials in storage.

The benefits go beyond these communities; however, extending into the environmental and medical fields. Improved analysis will help identify environmental soil and water samples contaminated by runoff and erosion from blasting and storage sites at lower levels, so the problem can be remedied before permanent damage occurs. Along with proper extraction techniques, GC/MS is capable of isolating and identifying each specific compound at the trace levels at which these compounds will most likely be detected. The challenges associated with this research project, the solutions to those challenges, and the resulting mass spectra for high explosives and related compounds for compilation into a user-searchable database will be presented.

Explosives, Gas Chromatography, Mass Spectrometry

A11 Electrophoretic Deposition of Nanoparticles and Nano-Structured Particles for Latent Fingerprints Detection on Different Surfaces

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After attending this presentation, attendees will be aware of the potential offered by Electrophoretic Deposition (EPD) of different kinds of nanoparticles and nano-structured particles in the development of latent fingerprints on a wide variety of porous and non-porous surfaces.

This presentation will impact the forensic science community by proposing for the first time the EPD technique in the development of latent fingermarks, and highlighting how nanotechnology and forensic sciences are becoming more and more intertwined.

Nanoparticles and nano-structured particles form the basis of several detection techniques for latent fingerprints and several reviews are yet available on this topic.[1,2]

Multimetal Deposition (MMD), for example, can be used for visualizing latent fingermarks on a wide range of porous as well as non-porous surfaces. MMD employs colloidal suspensions of gold nanoparticles, which preferentially deposit on fingerprint ridges, rather than in the valleys, through electrostatic and hydrophobic interactions.[3] Several works appeared later, aimed at enhancing the applicability of the MMD technique and trying to overcome its tedious experimental procedure, such as MMDII method, the use of functionalised and/or stabilised gold nanoparticles, and Single Metal Deposition (SMD).

Further nanoparticles and nanostructured particles employed for latent fingerprint detection include metal-oxides (such as TiO_2, ZnO, SiO_2, Fe_3O_4, and Eu_2O_3), sulfides, selenide, and tellurides, which were employed as pure powders or as fillers for nanocomposites in both Small Particle Reagent (SPR) techniques and dry powder dusting.

The goal of this presentation is to propose the use of electrophoretic deposition in place of or in combination with the aforementioned detection techniques based on the deposition of nanoparticles and nanostructured particles. EPD is a two-step electrochemical materials processing technique, usually carried out in a two electrode cell. In the first step, an electric field is applied between two electrodes and charged particles suspended in a liquid move toward the oppositely charged electrode (electrophoresis). In the second step, the particles accumulate at the deposition electrode and create a relatively compact and homogeneous film (deposition). EPD possesses several advantages over more conventional ceramic production strategies like short formation time, simplicity of the experimental apparatus, and almost no substrate shape restriction. Moreover, EPD can be applied to any solid that is available as a fine powder or as a colloidal suspension, including metals, polymers, ceramics, and glasses.

It is apparent that EPD techniques can be inserted into a latent fingerprint detection sequence. In this presentation, for example, it will be shown how aqueous EPD can play an innovative role after the first step of MMD. As known colloidal gold suspension creates a thin conductive layer perfectly reproducing the positive pattern of the

fingerprint ridges. EPD can exploit this newly formed fingerprint-electrode to deposit a wide range of different ceramic nanoparticles in order to improve the contrast by changing the substrate background coloration.

It will be shown that electrophoretic deposition alone can also be exploited for latent fingerprint detection on metallic and semi-conductive surfaces. Indeed, the presence of a fingerprint residue alters the electrical conductivity of the substrate underlying the ridges. Thus, the effectiveness of the deposit will reflect this electrical discontinuity. In this framework, EPD may also be considered a valid alternative to vacuum metal deposition (VMD).

Several examples will be presented including the use of different ceramic nanoparticle suspensions on different porous and non-porous substrates. Preliminary results regarding the role of nanoparticles' morphology on their adhesion to fingerprint ridges, under the application of an applied electric field, will be presented as well.

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Latent Fingerprints, EPD, Nanoparticles

A12 Classification of Smokeless Powders by Cluster Analysis

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The goal of this presentation is to identify statistically valid sub-classifications of smokeless powders.

This presentation will impact the forensic community by providing a statistical basis for identification of smokeless powders beyond single- and double-base designations.

The purpose of this research is to determine if sub-classes (clusters) can be identified within two broad classes of smokeless powders, namely those that are single-based or double-based. Similarities between the total ion spectrum (TIS) were calculated and dendrograms of the similarity data were generated. Cophenetic correlation and inconsistency coefficients were calculated in order to determine the number of clusters which were present among the samples.

In 2009, the National Center for Forensic Science (NCFS) in collaboration with the Technical Working Group for Fire and Explosions (TWGFEX) developed a smokeless powders database, which consists of a compilation of analytical data for commercially available smokeless reloading powders. Methods utilized for analysis of these powders include stereomicroscopy, Fourier-Transform Infrared (FTIR) spectroscopy, and Gas Chromatography-Mass Spectrometry (GC/MS).

For this study, the GC/MS data for 87 smokeless powders analyzed in-house was exported as a condensed data file (CDF) format, and were used to generate the TIS. The TIS was generated by averaging the mass spectra across the entire chromatographic profile for each of the samples. The intensity values for m/z 43-400 were normalized to sum to one, and this data was used to calculate the similarities between the samples. Similarities that approach one indicate that the samples are more similar, while those that depart from one indicate that the samples are less similar.

Agglomerative hierarchical clustering was used to analyze the similarity data, and a study was done in order to determine the most appropriate distance (i.e., Euclidean, Manhattan, etc.) and linkage (i.e., Centroid, Complete, etc.) clustering method for further data analysis. The cophenetic correlation coefficient was calculated for each distance and linkage combination. Cophenetic correlation coefficients closer to one indicate that the clusters in the data, as defined by the dendrograms, closely reflect distances between objects in the original data. Agglomerative hierarchical clustering allows for the determination of natural divisions that may be present within data. This method initially assigns each object to its own cluster and continues by merging objects and/or clusters together until all samples are merged into one cluster. Natural divisions (i.e., optimal clusters) are determined based on the inconsistency coefficient. The inconsistency coefficient compares the link height in a given cluster hierarchy with the average of the link heights directly below it. A link that is approximately the same height as the links below it is said to be consistent, indicating that there are no natural divisions within the data. On the other hand, a link that has a significantly different height from that of the links below it is said to be inconsistent, which may indicate natural divisions within the data.

Preliminary results indicate that average linkage and correlation distance, as well as centroid linkage and correlation distance, methods are the most suitable for analysis of the smokeless powders data. The cophenetic correlation coefficient in both cases was 0.96, and the data clustered into two primary groups, namely single-based and double-based. In addition, there are a number of sub-clusters apparent within the two main groups. Some sub-clusters correspond primarily to a single manufacturer, while others are predominantly populated by powder kernels of a given shape. Additional investigations are underway to better understand the chemical nature of these sub-clusters.

Clusters, Similarity, Dendrograms

A13 Development of a Chemically Relevant Artificial Fingerprint Material

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After attending this presentation, attendees will be aware of the development of a novel artificial fingerprint material, comparing to both human fingerprints and other artificial fingerprint materials, and discussing the use of mass spectrometry techniques to qualitatively compare the composition of both natural and artificial fingerprints.

This presentation will impact the forensic science community by providing a potential method for developing a chemically relevant artificial fingerprint material and a number of different possible applications in which the use of artificial fingerprint material could be used.

The main focus of this work was development of an artificial fingerprint material to provide a reproducible sample for completing fingerprint aging studies as well as degradation studies; however, a comprehensive artificial fingerprint material could also be used in understanding the mechanism of development techniques, evaluating the efficiency of developing techniques, and understanding the interactions of fingerprint materials with various surfaces.

Human fingerprints are generally composed of two types of secretions – eccrine and sebaceous. The eccrine secretions, which are almost completely composed of water, also contain a number of other components including salts, amino acids, lactic acid, proteins, and vitamins. Sebaceous secretions contain chemicals such as free fatty acids, cholesterols, esters, and glycerides. While it would be extremely difficult to replicate the entire chemical composition of a human fingerprint, it is possible to develop an artificial fingerprint material containing a number of the most abundant and chemically relevant fingerprint components.

In this work, a series of mass spectrometry based techniques – including Secondary Ion Mass Spectrometry (SIMS), Direct Analysis in Real Time Mass Spectrometry (DART®-MS), and Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) have been used to characterize real fingerprints and identify individual compounds typically found in fingerprints. These components were then compared

to those discussed in literature. From the results of these analyses, several iterations of artificial fingerprints, containing either or both sebum and eccrine secretions, were prepared. Every iteration was then compared, using multiple mass spectrometry techniques, to the collection of spectra from human fingerprints. Furthermore, the artificial fingerprint material was also compared to a number of other artificial fingerprint materials, both commercially available and reported in literature. Finally, once a chemically relevant artificial fingerprint material was developed, a way to reproducibly deposit the material was attempted. Initial studies are being completed to evaluate how piezoelectric inkjet printing can provide a way of accurately controlling the amount of material deposited on a surface of interest. The printing technique has the ability to print single spots or arrays of spots in a range of masses and possibly without the need of dissolving the fingerprint material in a solvent.

The ability to make a chemically relevant fingerprint material can have wideranging applications. It can allow for a reproducible sample set for any type of latent fingerprint research. The ability to deposit the fingerprint in spatially selected areas using inkjet printing could also be extremely useful for certain applications.

Fingerprints, Sebum, Mass Spectrometry

A14 Percutaneous Absorption of Organic Gunshot Residue Associated With Polymeric Membranes Using Ion Mobility Spectrometry

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After attending this presentation, attendees will gain an understanding about dermal absorption principles and how the dermal absorption of organic gunshot residue (GSR) can be used in gunshot residue casework studies.

This presentation will impact the forensic science community by highlighing potential uses of Ion Mobility Spectrometry (IMS) for the detection of organic gunshot residue that has been transferred to human skin after the use of a firearm and has been percutaneously absorbed. IMS analyses are fast, inexpensive, and generate a minimum amount of waste. The data showed that the method could play a valuable role in the definitive analysis of gunshot residue in casework samples.

Smokeless powders are a class of propellants that were developed in the late 19th-century to replace black powder. Organic additives, such as diphenylamine (DPA), are used as stabilizers and are also present in gunpowder composition. DPA stabilizes the energetic composition by binding nitrous oxide gases that have originated from the decomposition of nitrocellulose and converts them into stable compounds. Identification of stabilizers like DPA has become of importance in forensic science because it can provide valuable evidence in firearm discharge cases. Additionally, DPA is frequently used in the construction of improvised explosive devices (IEDs) related to criminal and terrorist acts.

Previous work has demonstrated that DPA can be detected on the skin of persons who have fired a weapon for about four hours, after which the concentration of DPA and other organic residues drops below IMS detection limits. These loses are not due to secondary transfer, which suggests that at least some portion of the compounds are being absorbed into the skin. To test this hypothesis, preliminary dermal absorption studies in the laboratory using polymeric membranes have shown that DPA possesses penetrating properties. The cumulative amount of DPA that permeated through the membranes increased over the course of 24 hours, which correlated well with the aforementioned persistence study. The calculated values of three key dermal absorption parameters were as followes: steady-state flux (J_{SS}, $\mu g/cm^2/hr$) 5.6 ± 1.7, permeability coefficient (K_p, cm/hr) $2.9 \times 10^{-3} \pm 8.5 \times 10^{-4}$, and lag time ($\tau$, hr) 1.1 ± 0.7. These results are comparable with the calculated values using the Potts and Guy skin permeation equation calculator available on the National Institute for Occupation Safety and Health's (NIOSH) website. The goal of this study was to build upon the DPA results and expand into evaluating the dermal absorption of other organic compounds present in gunshot standards and gunshot residue samples. Ion mobility spectrometry (IMS) was used for the qualitative analysis of organic gunshot residue for *in vitro* transdermal Franz diffusion cell (FDC) experiments.

IMS analyses were performed using an IONSCAN-LS™. IM station software (version 5.389) was used for data acquisition and processing. For the analysis of organic gunshot residue samples, the sample introduction into the IMS was performed through thermal desorption on a Teflon membrane. Organic gunshot residue was analyzed in the positive ionization mode while nicotinamide served as the calibrant. To simulate human skin, polydimethylsiloxane (PDMS) membranes (0.006" thick), were used for *in vitro* release testing. The membranes were the appropriate size to fit the FDC diameter (1" o.d) and their surface areas were 0.64 cm^2. Organic gunshot standards and gunshot residue samples were applied to the surface of the PDMS membranes and sampling occurred at regular time intervals. The cumulative amount of DPA found in the receptor fluid (μg) was plotted against time (hr). Linear regression was performed on the steady-state part of the curve to calculate the steady-state flux, skin permeability, and lag time.

In conclusion, results to date indicate that organic compounds associated with GSR are absorbed dermally and as such could be developed into another avenue for making a shooter/non-shooter decision based on chemical evidence.

Ion Mobility Spectrometry, Diphenylamine (DPA), GSR

A15 The Development of Field Calibrants for Detection Canines

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After attending this presentation, attendees will learn the odors associated with illicit materials as well as the validation strategy of surrogate continuation aids that can be used to train biological detectors, such as canines.

This presentation will impact the forensic community by demonstrating how a detection canine can be as objective and reliable as a laboratory instrument.

The goal of this study was to aid in the standardization of detection canine training and handling through the use of field calibrants. Field calibrants, or surrogate continuation aids, are becoming more common in the field since obtaining and maintaining detection canine training aids can be problematic and require adherence to rules and regulations that may be beyond the capabilities of the agency maintaining the canine team. Ideal field calibrants overcome these commonly encountered challenges by having inert compositions not subject to the Bureau of Alcohol, Tobacco, Firearms and Explosives (ATF) and Drug Enforcement Administration (DEA) terms and conditions. Field calibrants are also advantageous because they can be selected and manufactured in such a way that a known and controlled quantity of odor is released with minimized contamination concerns ensuring standardized training aids over their specified lifetime.

Biological detectors such as canines are valuable tools used for the rapid identification of illicit materials because they can be trained to reliably detect a wide variety of odors. However, recent increased scrutiny over the reliability of detection canines is currently being evaluated in the legal system as there are no formal regulations regarding detection canine maintenance and training. Best practices guidelines covering the various aspects of canine and orthogonal detectors for consistent and optimal practice among canine handlers have been established by the Scientific Working Group on Dog & Orthogonal Detector Guidelines (SWGDOG). SWGDOG has also outlined areas of needed research including the identification of odors associated with illicit materials and the validation of surrogate continuation aids, both of which are addressed in this study.

Previous studies have identified the dominant odor compounds of several illicit materials, which has led to the development of surrogate

continuation aids. Dominant odor compounds can be defined as the chemical compound or compounds identified in the headspace of the illicit material which, when exposed to the biological detector, induce an alert response. For example, methyl benzoate and 2,4-dinitrotoluene have been identified as the dominant odor compounds of cocaine and trinitrotoluene (TNT), respectively. This study deals directly with the steps taken for the validation of surrogate continuation aids and a Universal Detector Calibrant (UDC). Validation steps for these field calibrants include: the identification of the dominant odor compounds of the illicit material, the development of a surrogate continuation aid, field testing the surrogate continuation aid using trained and certified detection canines, substitution of the surrogate continuation aid into the initial training stages of the detection canines, and finally, the implementation of the surrogate continuation aid into daily training.

The development of a UDC provides one measure to evaluate the reliability of the biological and instrumental detectors. Currently there are no set practices to ensure that a biological detector is working at a reliable and suitable level on a daily basis. As instruments in a laboratory are calibrated to ensure that they are in proper working order, developing a UDC for which biological detectors can be calibrated would be useful. By training the canine to alert to the UDC before each working day, the handler can record if the biological detector is working to a suitable standard. The UDC has the potential to also be used in selecting future biological detectors by determining the time it takes to train the canine to alert to the compound and the sensitivity of detection that the canine can achieve. Standardization of detection canine training aids will ensure the optimal number of illicit material odors detectable in the most reliable manner. Implementation of field calibrants into daily detection canine training will improve the reliability of the canine by allowing for the direct comparison of other biological detectors as well as orthogonal detectors.

The Science and Technology Directorate of the U.S. Department of Homeland Security partially sponsored the production of this material under Interagency Agreement IAA # HSHQDC-10-X-00297 with the National Institute of Standards and Technology (NIST). Additionally, this research was supported in part through a Doctorial Evidence Acquisition Fellowship awarded by Florida International University.

Detection Canines, Training Aids, Calibration

A16 False Negatives and Decreased Sensitivity of Heme Tests on a Leather Substrate: Incidence and Causes

Alex J. Krotulski, BS, Kei A. Osawa, BS*, and Anna S. Duggar, MS, Loyola Univ, Dept of Chemistry, 6363 St Charles Ave, New Orleans, LA 70118*

After attending this presentation, attendees will be made aware of the potential for presumptive and confirmatory false-negative test results as produced by bloodstains on leather casework samples, and the possible chemical causes for the desensitized reaction.

This presentation will impact the forensic science community by showing how the possibility of substrate interference has significant implications. Although there are many publications devoted to the possibility of false positives when testing for blood (one of the most recent being a presentation at an AAFS meeting in 2011), comparatively little time has been spent investigating false negatives. Leather boots, jackets, and upholstery are encountered regularly as substrates during the investigation of violent crimes, and in this study, are identified as a possible source of false negative screening results. In turn, these results would mean lost opportunities for more discriminatory testing, such as DNA analysis. Field and serology analysts may be able to use the information generated during this study when deciding whether to include a "negative" sample for further DNA analysis.

Anecdotal reports from the New Orleans Police Department Crime Lab suggested that blood samples on Timberland® boot leather may produce false negative results when using phenolphthalein, a heme-catalyzed presumptive blood test. This claim was tested by acquiring boot leather samples and applying serially diluted human blood, using cotton cloth as a comparative standard. A general decrease in sensitivity over time, greater than that observed on control samples, was observed in the boot leather samples: in less than two weeks, blood dilutions of $1/10^3$ were still detectable on cotton cloth, but only 1/10 dilutions and whole blood were detectable on Timberland® boot leather. The result was then duplicated in upholstery leather samples when, after only two weeks, phenolphthalein sensitivity decreased from $1/10^3$ on cloth to 1/10 on two different types of upholstery leather. The common decrease indicates that the chemical inhibitor is most likely not specific to Timberland®-brand tanning processes, but is common to the leather industry as a whole.

A similar order-of-magnitude decrease in detection of blood on both boot leather and upholstery leather (as compared to cotton substrates) was observed with benzidine (another heme-catalyzed presumptive blood test); with Hematrace antibody/antigen test kits; and with traditional Takayama crystal testing. Heme-catalyzed color tests and Hematrace human hemoglobin detection tests showed a decrease in sensitivity in as little as two weeks; Takayama crystal tests showed a decrease in sensitivity in under a month.

The three tests utilize varying elements of the hemoglobin molecule. In heme-catalyzed color tests, the heme group central to the hemoglobin molecule behaves as a peroxidase, reducing hydrogen peroxide to water and, in turn, depleting the hemoglobin of electrons; these electrons are replaced from the test dye molecule, transforming the molecule from its colorless to a colored state. Although these tests are known to be pH-sensitive, phenolphthalein and benzidine have substantially different effective ranges (between 8 and 10 versus any pH above 4). The ABAcard Hematrace test uses anti-human-hemoglobin antibodies to produce a highly sensitive color-positive result. The Takayama crystal test relies on the formation of pyridine hemochromogen crystals when the reagent is reacted with hemoglobin and heated. As all three of these tests displayed reduced sensitivity and detection potential when performed on bloodstains exposed to a leather substrate, the question remains as to what chemical aspect of the leather substrate is affecting the tests, and whether the reaction can be easily reversed.

As the undesired effects appear not to be pH-dependent, and are common to multiple types of leather, the presence of residual tanning chemicals is the most likely source of the observed false negative effects. Further study on the effects of tanning chemicals on the iron atom at the center of the heme moiety is expected to reveal the source of the multi-test false negatives. Whether or not a simple counter-treatment can be identified, criminalists should be mindful of the age and environmental history of leather case samples submitted for serological screening, and make analytical decisions accordingly.

Blood Detection, Heme, Leather

A17 Advances in the Forensic Use of Human Scent

Lauren J. Colon-Crespo, BSc, Jessica S. Brown, BS, Norma I. Caraballo, BSc, and Kenneth G. Furton, PhD, Florida Int'l Univ, Dept of Chemistry and Biochemistry, 11200 SW 8th St, CP344, Miami, FL 33199*

After attending this presentation, attendees will increase their understanding of the sample collection procedures and analytical methods used to evaluate human scent from different biological specimens.

This presentation will impact the forensic science community by providing an overview of several recent research studies that have examined human scent, demonstrating its importance and application for the criminal justice system.

In 2010, Joshua Wade was sentenced to life in prison for the 2007 carjacking and murder of his neighbor Mindy Schloss. Human scent evidence played a crucial role in the resolution of this case after FBI-trained canines were able to identify both the victim's and Wade's scent on different items, as well as follow scent trails linking several locations and pieces of evidence to Wade. In *California vs. Benigo Salcido,*

challenges arose regarding canine evaluations of human scent, such as the durability of human scent, the ability of canines to discriminate accurately between scents, and the use of human scent to distinguish different individuals.[1] The courts found that human scent discrimination by a canine can be admitted into court as evidence whenever the training and experience of both the dog and the handler prove to be proficient, the handler's methods prove to be reliable, and the person performing the sample collection technique uses the correct scientific procedures. These court cases have highlighted the significance of human scent within the criminal justice system and in forensic investigations.

This presentation will summarize and discuss human scent research and its ability to be used as a form of associative evidence in forensic investigations. Presently, human scent-discriminating canines are often employed to discriminate individuals, crime scenes, and objects. This practice is based upon the theory that every individual possesses a distinct odor that is generated from a complex combination of the body's metabolism, gland secretions, hormonal control, and interactions with the residing bacterial populations.[2] Human scent is comprised of secretions and bacterial action that occurs on dead skin cells, commonly referred to as "rafts", which can easily be deposited into the environment allowing canines the opportunity to pick up a person's scent.[3] To obtain an understanding of what canines smell, human scent has been studied in the laboratory using a variety of specimens. A large portion of research has been conducted on hand odor since there is a high likelihood that a suspect's hands will come into contact with an object while committing a crime. Hand odor can be collected through two different methods: (1) direct contact of the collection material and the object/person; and, (2) non-contact sampling using a portable vacuum source that is designed to draw in volatile organic compounds (VOCs) from the object/person. Upon collection, scent samples are analyzed using solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS) and statistically evaluated to determine the distinguishability of the scent profiles obtained from different individuals.

Human scent research has advanced over the years to include other biological specimens of forensic interest, such as hair, nails, and saliva. Brown and Furton found that hair, nails, and saliva possessed VOCs that differed between specimens and between people, allowing for the discrimination of individuals. This was further evaluated using human scent-discriminating canines.[4] Field tests revealed these specially trained canines had the ability to discriminate an individual independent of the specimen being assessed.[5] In another study conducted by DeGreeff *et al*. in 2011, human scent profiles of living individuals were compared to those that were deceased.[6] It was found that human scent profiles of living subjects differed from one individual to the next; however, upon death, the scent profiles appeared similar from one person to the next, revealing a more general odor for deceased individuals. This presentation will highlight and explore the advances in human scent research, which demonstrates its significance, versatility, and application in forensic investigations and within the criminal justice system.

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Human Scent, VOCs, Canines

A18 Testing a Combined Approach for DNA and Trace Evidence Recovery Using Tape Lifting on Forensically Important Substrates

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After attending this presentation, attendees will have been introduced to a novel approach for recovering DNA and various types of trace evidence from forensic samples, specifically, a common technique called tape lifting, to collect both trace evidence and "touch" DNA across multiple substrates using modern, automated methods of forensic analysis.

This presentation will impact the forensic science community by promoting a comprehensive approach to technique evaluation, expanding awareness to other instances of discipline overlap, and exploring the effects of other methods on common trace practices. A single type of tape that is optimal for trace evidence and touch DNA collection would streamline training, further interdisciplinary cooperation, and promote synergy during evidence routing and analysis in the forensic science laboratory.

The goal of this study is to test differences in recovery efficiency for trace evidence touch DNA across six types of tape currently in use or being considered for use in the Ventura County Sheriff's Office Bureau of Forensic Services: Evercare™ Lint Pic-Up roller, 3M Scotch™ Lint Roller, HP-260 Duck™ Brand Packing Tape, Lyn-Peavey™ Fingerprinting Tape, Allene's® Tacky Double-Stick Sheets, and Staples® Stickies™ Adhesive Notes. All tapes were tested for the presence of (PCR) inhibitors based on the quality of their internal PCR controls after quantification and the appearance of their electropherograms after amplification. The inhibition study was performed with xylene-treated swabs, water-treated swabs, and with no swabbing of the tape. DNA was provided by spotting 10 µl of 1:200 diluted whole blood from a single source onto the tape to give 3ng of DNA. The ability to recover touch DNA was determined by comparing the average quantifiable DNA from each tape to the average DNA from three, co-extracted reference swabs after tape lifting dried saliva stains from tight weave, knit, and glass substrates. Saliva stains were produced by pipetting 15µl of a 1:30 dilution of whole saliva onto the substrate to give 15ng of DNA. Finally, trace evidence recovery for each tape sample was examined by comparing tape lifting efficiencies of glass fragments, four-layer house paint chips, 100% polyester clothing fibers, and 100% nylon carpet fibers, which had all been rendered to 0.5mm as well as whole pubic hairs and 100% nylon carpet fibers from the same three substrates. Quantitation of touch DNA was carried out using a sequence detection system with an amplification kit and amplified with an amplification kit and an genetic analyzer capillary electrophoresis instrument. Trace evidence identity was established with FT-IR using a single-bounce attenuated total reflectance accessory.

All tape samples produced fully amplified and interpretable profiles across all Combined DNA Index System (CODIS) loci with little to no PCR inhibition, regardless of the pre-extraction treatment. The Duck® Brand HP260 High Performance Packaging Tape recovered the highest amounts of trace evidence and touch DNA across all three examined substrates. While the packaging tape was transparent and easy to

analyze, it also picked up much of the background material present in or on the substrate and was somewhat difficult to use compared to the other tapes. The 3M Scotch™ Lint Roller was consistently the second best tape when recovering every type of trace evidence (except 0.5mm carpet fibers) and touch DNA while being easier to use on bedding and other large surfaces. In spite of its difficulty collecting small nylon carpet fibers, the 3M Scotch™ roller never lagged more than 10% recovery behind the packaging tape. All other tapes performed well in specific circumstances, but had inferior recovery efficiency compared to both the packaging tape and the lint roller across all three substrates. The Duck Brand® packaging tape was suggested as the optimal tape for the Ventura County Sheriff's Office Bureau of Forensic Services while the 3M Scotch™ was advised to be used for large areas or in case the packaging tape was not available.

Tape Lifting, Touch DNA, Trace Evidence

A19 Identification of Sildenafil and Other Male Potency Enhancers Found in Internet Products

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After attending the presentation, attendees will understand that erectile dysfunction pharmaceutical medication is being put into Internet products that are being marketed as all natural or herbal remedies. Attendees will be shown data from various analytical techniques such as Thin Layer Chromatography, Gas Chromatography/Mass Spectrometry, High performance Liquid Chromatography/Ultraviolet Detection and Liquid Chromatography/Mass Spectrometry. They will be made aware that this phenomenon is happening in the United States and around the world. Finally, they will be shown dangerous physical effects that can occur by taking these products without a prescription from a physician.

This presentation will impact the forensic science community by showing that the adulteration of Internet products with Viagra© and related compounds is actually occurring. This is significant because these medications have dangerous side effects and various contraindications, and should not be taken without a doctor's prescription. Many members of the forensic science community may not be aware this adulteration is happening; therefore, it is extremely important this information is made available to the community.

There has been an increase over the last decade in the sale of products marketed as male potency enhancers that can be purchased through the Internet. Erectile dysfunction drugs that are only available by a doctor's prescription such as sildenafil (Viagra©), tadalafil (Cialis©), and vardenafil (Levitra©), or their chemical analogs, are being added to various Internet products proposed to naturally enhance male potency, without regulation or medical approval. The danger in this manifests itself in the various side effects of these drugs that include stroke, loss of vision/hearing, and death. These drugs act as specific phosphodiesterase type 5 (PDE-5) inhibitors that block the degradation of an enzyme that allows for increased blood flow to the penis, thus maintaining an erection. The enzyme involved is cyclic GMP and has a variety of functions in the body, including being a carrier protein. This, in turn, causes significant vasodilation and can lower blood pressure drastically. Men with cardiovascular problems would normally not be prescribed this medication due to the inherent risks; however, now they have the possibility of obtaining it without a doctor's prescription by buying products on the Internet, in local shops, or in gas stations.

Various unregulated erectile dysfunction products were ordered over the Internet and analyzed by Thin Layer Chromatography, Gas Chromatography/Mass Spectrometry, High performance Liquid Chromatography/Ultraviolet Detection, and Liquid Chromatography/Mass Spectrometry. Ten of these products were examined. The matrices explored were pills, powders, and even a cream. They were all purchased over the Internet for a very low price and all were marketed as "all natural." Ingredient lists made no mention to the presence of sildenafil, tadalafil, or vardenafil. Results obtained indicate the presence of these compounds in various Internet products. Furthermore, other compounds such as caffeine were also found in these products without being listed on the ingredient list. This indicates that companies, both in the United States and overseas, are falsely marketing these products and selling them over the Internet, making them very accessible. This threatens the safety of individuals that may experience damaging side effects while consuming these products, believing them to be all natural.

Sildenafil, Internet Products, Adulteration

A20 Optimized Screening of Synthetic Cannabinoids With Phenyl Reversed-Phase Liquid Chromatography

Kaitlin Giovanni, BS, Mercurio Veltri, PharmD, William F. Nirode, PhD, and Ling Huang, PhD, Hofstra Univ, Chemistry Dept, 151 Hofstra University, Hempstead, NY 11549*

After attending this presentation, attendees will understand the fundamental principles of Reversed-Phase Liquid Chromatography (RPLC) with UV-Vis detection as pertaining to forensic chemistry, and the necessary elements for optimized separation and identification of illegal substances, specifically synthetic cannabinoids in herbal incenses. Through the comparison of several different phenyl-functionalized columns, the optimal screening technique for synthetic cannabinoids can be determined and used in a practical setting.

This presentation will impact the forensic science community by providing an optimized and simple chromatographic separation and quantification method for synthetic cannabinoids in herbal incenses in order to improve the accuracy and throughput of "spice" evidence processing.

The recently-passed S.3187 included five classes of synthetic cannabinoids into Schedule I controlled substance list. It creates great challenges for forensic scientists to rapidly separate, identify, and quantify these "cannabimimetic agents" in numerous herbal incenses due to the similarity of isomer or analog structures. Newer compounds are being synthesized promptly to circumvent the ban, which exacerbates the analytical difficulties for forensic labs, some of which are already experiencing backlogs. In this presentation, a novel yet simple RPLC separation method with phenyl functionalized column and Diode Array Detector (DAD) was dicovered to possess several advantages over conventional C-18 LC/MS methods. When the phenyl RPLC method is coupled with a simple liquid extraction method, the identification and quantification of synthetic cannabinoids in herbal incenses can be completed in less than 30 minutes.

All cannabinoids contain aromatic functional groups such as phenyl, naphthyl, indole, and pyrrole, which readily interact with phenyl stationary phases through pi-pi interactions. Compared to conventional C-18 or C-8 columns, these phenyl columns provide greater resolution in the separation of similar cannabinoids. Six different phenyl columns were compared to determine the ideal separation conditions for synthetic cannabinoids. The injection volume of the sample, composition of the mobile phase, and temperature are optimized to achieve ideal resolution and speed. A mixture of ten synthetic cannabinoids (6-16µg/mL methanol), including JWH-122, AM-2201, RCS-4, and various isomers and analogs, were prepared to evaluate the separation efficiency. For the herbal incense samples, a simple methanol extraction with paper filtration was employed as LC sample preparation. All separations were carried out isocratically on a HPLC system with pure acetonitrile and water as mobile phases flowing at 1-1.5mL/min. The detection wavelengths were set at 214nm and 280nm to capture all synthetic cannabinoids in 1-10µL injection volumes. Elevated temperatures (50-60˚C) increased separation speed.

After the optimization of separation conditions, all six columns can separate seven out of ten cannabinoids with satisfactory resolution in under 20 minutes. Phenylhexyl columns provide better separation compared to phenylpropyl or biphenyl columns, thanks to the diverse functional groups presented on the stationary phase surface and in the

analytes. Compact UPLC-type columns with porous shell provide more surface area in a shorter column, which increases speed without the sacrifice of resolution. Polymer reversed phase columns provide more uniform distribution of phenyl groups on the stationary phase, which performed better than silica-based phenyl columns. One polymer-based phenylhexyl column separated all 10 standards in under 14 minutes. With standard calibration, the synthetic cannabinoids in 30 samples were identified and quantified. Most herbal incense contain two to three cannabinoids with concentrations varying from 1-20mg/g of herb.

In conclusion, phenyl RPLC columns with DAD can be used to quickly separate, identify, and quantify synthetic cannabinoids in herbal incenses. When coupled with a simple methanol extraction method, the isocratic separation provides better resolution and rapid quantification compared to conventional C-18 LC/MS methods.

Cannabinoids, Chromatography, Drug Analysis

A21 Statistical Measures for Comparisons of Fiber Spectra: Forensic Database and Statistical Software

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After attending this presentation, attendees will be acquainted with the utility, ease of use, and applicability of a forensic fiber database containing fiber characteristics and user-friendly software for visual and statistical comparison of fiber spectra. These combined tools offer support for objective data-based decisions regarding the similarity of questioned and known fiber data and the reliability of match exclusions.

This presentation will impact the forensic science community by describing a combined data archiving and statistical graphics and analysis system that offers both data management and decision-making support to the forensic fiber examiner. Trace fiber investigations, where the hypothesis of a common source for two fibers could not be rejected and evidence was found to have probative value.

Testimony of FBI examiner Paul Stombaugh before the Warren Commission concerned some fibers stuck on a jagged edge of Oswald's rifle stock stated, "There is no doubt in my mind that these fibers could have come from this shirt." However, the next statement, "There is no way; however, to eliminate the possibility of the fibers having come from another identical shirt," epitomizes the problem of class evidence. The goals of this research include: (1) assess error rate performance in trace fiber evidence examinations based on UV/visible microspectrophotometry and infrared spectroscopy; (2) create a database of fiber characteristics, including UV/visible or IR spectra, to establish a performance baseline relevant to discussions of fiber discrimination; and, (3) evaluate intra- and inter-laboratory consistency, and improvements in forensic laboratory practice.

Statistical evaluation of trace evidence data from UV/visible or IR spectroscopy has great utility for assessment of assessing similarity or dissimilarity of spectra when comparing questioned and known trace evidence samples. The use of statistical hypothesis testing with both univariate and multivariate data, coupled with good experimental design, permits the investigator to exercise control over errors in statistical decisions arising from measurement uncertainties. Both univariate and multivariate statistical methods to judge the significance of discrimination of UV-visible and infrared spectra from a wide variety of textile fibers were employed. Statistical methods, coupled with informative graphics for comparing grouped data distributions, can produce statistical support for inclusion and exclusion decisions in forensic fiber examinations. This presentation will show statistical analyses of fiber spectra from a user-friendly software package that has been developed for this purpose. The second tool presented here is a forensic fiber database that facilitates archiving fiber data such as polarized microscopy measurements (birefringence, sign of elongation), physical characteristics (diameter, shape), and spectral data. One immediate advantage is the ability to store data in a documented manner and access this information on demand from a relational database. XML, an extensible markup language that can be employed to define a flexible and self-documenting, but human-readable and standardized text format for forensic data was adopted. XML has experienced recent growth because of its adoption by a multinational software corporation as a file format for office application documents. NIST has long recognized the significance of XML and several ongoing interagency efforts involve data format standardization using XML (e.g., the NIST ITL American National Standards for Biometrics and efforts by ANSI, NIST, and the FBI). The web-based SQL/ASP database currently holds information on about 500 fibers, and facilitates queries for rapid retrieval of data and interactive visualization, export of existing raw data, and import of new fiber data sets. Although there is little likelihood of establishing a truly comprehensive fiber database because of fast-moving trends in manufacturing and globalization of production, a combined data archiving and statistical graphics and analysis system offers both data management and decision-making support to the forensic fiber examiner.

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Fiber Spectra, Statistics, Database

A22 In-House Production of a Metrologically Sound Traceable Ethyl Centralite Reference Material for Smokeless Powder Analysis

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After this presentation, attendees will be able to rationalize the in-house production of a metrologically sound, traceable, high purity Ethyl Centralite (EC) reference material for smokeless powder analysis and extend this knowledge to other compounds.

This presentation will impact the forensic science community by providing a method for analysts to make their own reference materials required in their routine analyses in a metrologically sound, traceable manner in order to compensate for the lack of available forensic reference materials. The chemical compound chosen to demonstrate this approach was EC, a common organic stabilizer of smokeless powders.

The analysis of organic compounds present in smokeless powder from ammunition and improvised explosive devices is important in crime elucidation, especially after the advent of "environmentally friendly" ammunition, free of barium, lead, and antimony. The most common organic additive in Brazilian ammunition is EC, in which its function is to stabilize the smokeless powder by delaying degradation of nitrocellulose. Both qualitative and quantitative analysis of additives and propellants in smokeless powder are important for forensic scientists. By qualitatively identifying and quantitatively determining the additives in smokeless powder it is possible to calculate a numerical propellant to stabilizer ratio which allows the association of handgun-fired organic gunshot residues with unfired powder. To perform quantitative analysis of most Brazilian ammunitions, the use of a smokeless powder certified reference material (CRM) containing EC is recommended for calibration, method validation, and quality control. Workers at the Brazilian National Institute of Metrology, Quality and Technology - INMETRO are working to produce a smokeless powder CRM containing EC as additive. However, to quantify EC in smokeless powder for the production of such a CRM, or even to quantify EC in common routine analysis, a high purity

EC CRM is required for calibration. Because such a commercial CRM is also not yet available worldwide, in-house production of a metrologically sound, traceable EC reference material for smokeless powder analysis is necessary. The objective of this research was to assess the purity of a commercial EC in a metrologically sound, traceable manner.

High purity EC was purchased and the identity of EC was confirmed by determination of melting point, Mass Spectrometry (MS), Fourier Transform Infrared spectroscopy (FTIR), and 1H and 13C Nuclear Magnetic Resonance (NMR). The purity was assessed by three independent methods: Differential Scanning Calorimetry (DSC), quantitative 1H NMR (using benzoic acid NIST 650b SRM as internal standard), and mass balance. The latter consisted of a broad investigation of the impurities, which included identification and quantification of non-volatile organic impurities (by gas chromatography coupled to flame ionization detector – GC-FID), volatile organic impurities (by headspace GC-FID), water content (by coulometric Karl Fischer titration), and inorganic impurities (by ashing).

EC melting point was 72.57 ± 0.03°C. Mass spectra of sample showed m/z = 268, 164, 148, 120, and 77, which is consistent with EC structure. FTIR and 1H and 13C NMR spectra were consistent with EC structure. All these data confirmed the identity of the commercial product as EC.

EC purity by DSC was 99.83 ± 0.05 mol % (average and standard deviation). EC purity (m/m) by quantitative 1H NMR was 99.89 ± 0.7% (average and measurement uncertainty – 95% CI; k = 1.98). EC purity (m/m) by mass balance was 99.86 ± 0.02% (average and measurement uncertainty – 95% CI; k = 2). The mass balance is also called 100% - impurities % method and the individual impurities found were (m/m): (1) non-volatile organic impurities: methyl-ethyl centralite (0.0762%); (2) volatile organic impurities: m/p-xylene (0.0263%), ethyl benzene (0.0079%) and dimethylchloramine (0.013%); (3) water content: 0.0083%; and, (4) inorganic impurities: 0.0025%.

EC purity results by three independent methods are in agreement with one another. It was decided to consider the mass balance result (99.86 ± 0.02% m/m) because of the reduced measurement uncertainty valor. Metrological traceability came by the use of a primary method (DSC), by the use of a certified reference material in quantitative NMR, and by a detailed investigation of impurities, which provided an indirect purity assessment (mass balance). This approach can be used for the production of in-house reference materials suitable for calibration, quality control (after spiking a blank matrix), and method validation involving forensic analysis.

Reference Material, Smokeless Powder, Ethyl Centralite

A23 Presumptive Color Test for Piperazine Designer Drugs

Tsunghsueh Wu, PhD, Chelsea Johnson, BS*, and Ethan Becker, BS, Univ of Wisconsin, 1 University Plaza, Platteville, WI 53818*

After attending this presentation, attendees will understand the development of a presumptive color test for piperazine designer drugs, the scientific principles underlying drug detection, and the results on detection of BZP and TMFPP using this technique.

This presentation will impact the forensic science community by introducing the new technique for law enforcement to effectively conduct the drug test in the field.

Presumptive color tests for drugs help investigators to narrow the possible identities of a substance. It is a quick and inexpensive chemical test, which is commonly performed by police officers on the street prior to the use of costly confirmative tests in the forensic science laboratory. This test is done to determine quickly on the scene if the police officer has probable cause for an arrest. Using this new technique, an investigator places the questioned substance in a disposable test tube containing ampules of chemical reagents necessary for the presumptive identification of piperazine drugs such as benzylpiperazine (BZP) and 3-trifluoromethylphenyl-piperazine (TFMPP).

Benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP) are synthetic phenylpiperazine analogues, which have stimulant and amphetamine-like properties.[1] As a result, they are commonly used as a recreational drug, and were legally available in a number of countries, particularly in New Zealand.[1] The drug was temporarily classified as a Schedule I controlled substance in the United States in 2002 because of its high abuse potential and lack of accepted medical use or safety.[2] On March 18, 2004, the DEA published a Final Rule in the Federal Register permanently placing BZP in schedule I. Several states have placed BZP in schedule I: Colorado, Idaho, Illinois, Indiana, Iowa, Kansas, Louisiana, Mississippi, Missouri, Oklahoma, Nebraska, Tennessee, and Wyoming.[2]

According to Office of Diversion Control, law enforcement officials submitted 48 drug items/exhibits to federal, state, and local forensic laboratories identified as BZP in 2004.[2] The number of BZP items/exhibits increased from 437 in 2007 to 6,088 in 2008. BZP items/exhibits submitted to forensic laboratories increased 127% from 6,088 in 2008 to 13,822 in 2009. BZP was smuggled internationally as powder by drug trafficking organizations. The bulk powder is loaded into capsules and pressed into tablets. BZP is encountered as pink, white, off-white, purple, orange, tan, and mottle orange-brown tablets. These tablets bear imprints commonly seen on MDMA tablets such as housefly, crown, heart, butterfly, smiley face, or bull's head logos and are often sold as "ecstasy." BZP has been found in powder or liquid form packaged in small sizes and sold on the Internet.[2] Because of the increasing trend in the distribution of these substances, the presumptive test for piperazine drugs is necessary to allow law enforcement to identify the controlled substances on the street within a minute.

Both BZP and TFMPP appear as a colorless solution when dissolved in deionized water. In this research, the BZP and TFMPP aqueous solution in the presence of our reagents will yield a yellow solution as a positive result. However, organic molecules containing primary amine functional group caused a severe interference. This presentation will discuss the principle behind the method and future plans to study this test method.

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Presumptive Test, Party Drug, Benzylpiperazine

A24 Differentiation of South American and Domestic (U.S.) Crack Cocaine Through Headspace-Gas Chromatography/Mass Spectrometry (HS-GC-MS)

John F. Casale, BS, and Valerie L. Colley, BS, DEA, Special Testing Laboratory, 22624 Dulles Summit Ct, Dulles, VA 20166*

After attending this presentation, attendees will understand the methodology used to differentiate South American- and U.S.-produced crack cocaine.

This presentation will impact the forensic science community by providing a method by which South American- and U.S.-produced crack cocaine can be differentiated.

South American coca leaf-to-cocaine base processors have been making cocaine base in the form of "crack cocaine" for several years for distribution and consumption in South America. There had been no evidence of South American-produced crack cocaine being smuggled into the United States until recent U.S. interdiction efforts. This form of cocaine is typically produced by boiling crude cocaine base (obtained directly from coca leaf through traditional illicit processing methods), skimming off the water and impurities, and then allowing the cocaine base to solidify into the form of crack cocaine. In contrast, U.S. domestic

crack cocaine is typically made by the conversion of imported illicit cocaine hydrochloride into cocaine base by dissolving cocaine hydrochloride in hot water, converting it to cocaine base through the addition of sodium bicarbonate (or some other base), boiling the solution, allowing it to cool, and then removing the water after the cocaine base has solidified into crack cocaine. The presented research will show that, as a result of these production differences, South American crack cocaine can be distinguished from domestically produced crack cocaine. In addition, it will show that analysis of domestically produced crack cocaine can provide information on what solvents were used in the original cocaine hydrochloride processing in South America (i.e., prior to the cocaine base conversion process into cocaine hydrochloride).

Samples of cocaine base from South America (Colombia, Bolivia, and Peru) and the U.S. were analyzed by Headspace-Gas Chromatography/Mass Spectrometry (HS-GC/MS) to determine their solvent profiles. Analyses of the South American exhibits confirmed traces of low to high boiling hydrocarbons present in the crystal matrix. These residual solvents are due to the use of gasoline, kerosene, and diesel in the extraction process. In contrast, analyses of domestic crack cocaine exhibits show solvent profiles typically seen for cocaine hydrochloride exhibits, but at much reduced levels. The results demonstrate that South American crack cocaine is easily differentiated from U.S. domestic crack cocaine.

The correlation between cocaine hydrochloride solvent profiles and their corresponding domestic crack cocaine solvent profiles was also investigated. Samples of cocaine hydrochloride were analyzed by HS-GC/MS to determine what solvents were used in the clandestine cocaine base-to-cocaine hydrochloride conversion process. Each resulting cocaine hydrochloride sample was then converted to crack cocaine using the traditional domestic crack cocaine production method. Each crack cocaine sample was then analyzed by HS-GC/MS and its resulting solvent profile compared to its original cocaine hydrochloride solvent profile. In each case, the crack cocaine solvent profile contained essentially the same primary processing solvents found in the original cocaine hydrochloride, but at reduced levels. The levels of reduction varied by solvent but were typically one-half to one-tenth of the original levels. This data can provide valuable information on what solvents were used to produce the cocaine hydrochloride in South America prior to the production of domestic crack cocaine.

Currently, the DEA Special Testing and Research Laboratory routinely analyzes imported illicit cocaine hydrochloride samples to determine which solvents were used in the clandestine cocaine base-to-cocaine hydrochloride conversion by South American processors. This data provides the intelligence community with valuable information, which is used to monitor and, in some cases, control essential solvents used by clandestine laboratories. Analysis of domestic crack cocaine samples to determine which solvents were used in the clandestine cocaine base-to-cocaine hydrochloride conversion by South American processors will augment the strategic intelligence currently provided for solvent control and diversion efforts.

Forensic Science, Crack Cocaine, Occluded Solvents

A25 Comparative Study of Shotgun Spread Patterns

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After attending this presentation, attendees will learn how to interpert statistical data, a shotgun spread pattern, and how the muzzle-to-target distance can be determined.

This presentation will impact the forensic science community by allowing examiners to refer to a statistical model to approximate the muzzle-to-target distance at a crime scene instead of performing test fires for each shotgun found at a crime scene.

When a shotgun is fired, the pellets exit the barrel in a tight packet. As the pellets get farther from the shotgun, they start to spread out and cover a larger area when they impact. The farther the target is from the shotgun, the wider the spread pattern becomes. Given that the same gauge, choke, and shot size is used, one should be able to predict the spread of the pellets over time. In 1983, Heaney and Rowe used a linear regression to model pellet dispersion from shotguns.[1] In their research; however, only one spread pattern at each distance was presented. This does not account for any variation in the spread pattern that could occur at that distance. In order to produce an accurate statistical model that accounts for variation, a larger sample size must be used. This research also only covered one degree of choke and pellet size. Arslan *et al*, completed a similar study in 2011.[2] A larger sample size at each distance was used in this study, which allows their statistical models to be more accurate. In this research, the effect of various gauges, choke types, and pellet sizes were also studied. The Arslan group concluded that each factor results in a new model to estimate shooting distances.

In this research, the effect of choke and pellet size was studied using a 500 series shotgun. Measures of dispersion such as maximum radius, a 95% confidence interval of the maximum radius, bounded rectangle, and fitted ellipse were used to measure the dispersion patterns. The position of each pellet in a spread pattern was calculated using the x and y coordinates of each pellet with the origin set in the center of the spread pattern. The 95% confidence interval at each distance was calculated by taking the average maximum radius plus/minus the standard deviation of the maximum radius multiplied by a t-value for that sample size. The bounded rectangle was calculated for each spread pattern by taking the maximum x value minus the minimum x value and the maximum y value minus the minimum y value. The x and y coordinates of each spread pattern were also used to calculate the width and height, a and b, of a fitted ellipse that represents the smallest ellipse that covers all of the pellets in the spread. Models were developed to estimate the firing distance and to associate an uncertainty in each particular test condition.

In the past when shotgun spread patterns were found at a crime scene, examiners would have to perform test fires with the suspect weapon to find the spread patterns of that gun at various distances. These test spread patterns would then be compared to the pattern found at the crime scene so the distance could be approximated. This research would instead allow examiners to refer to a known spread pattern at a given distance, pellet size, and gauge. The 95% confidence interval will allow examiners to say that they are 95% certain that the suspect spread pattern falls within the range presented in the model at that distance. Although the examiner may wish to confirm that these distances are correct with the suspect shotgun, this would allow the examiner to have a starting point, and save time when reconstructing the crime scene.

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Distance, Spread Pattern, Mossberg 500

A26 Analysis of a New Synthetic Street Drug “Molly Plant Food” by Mass Spectrometry and Vibrational Spectroscopy

Nisha V. Patel, BS, 1514 Center Pointe Dr, Murfreesboro, TN 37130*

After attending this presentation, attendees will learn about the composition of the new synthetic drug “Molly Plant Food” as well as the

methods used for analyzing the synthetic drug. Interpretation of data acquired via pyrolysis-gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, and Raman spectroscopy will be presented. The analysis of this new synthetic drug will contribute to better understanding of this and other similar variations of this synthetic drug with different substituent groups among the cathinones. The methodologies and findings will contribute to future protocols for analyzing and assessing the toxicological effects of the banned synthetic stimulants substances.

This presentation will impact the forensic science community by providing an analysis of the the synthetic stimulant drug toxicology, the various kind of methcathinone commonly detected in these stimulant drugs, and the findings of methcathinone in the Molly Plant Food. This rapid, reliable method of analysis of synthetic stimulant substances will enable law enforcement authorities to easily identify the new drug and better understand the toxicological effects.

In recent years, the production of fake "Ecstasy" has grown quite popular. Many abuse this new synthetic drug as a substitute for the real Ecstasy, which is illegal to possess and expensive to purchase in the black market. Fake Ecstasy is commonly known as Molly Plant Food or Rave Bath Salts is sold in convenience stores as common household products like bath salts or plant food.[1] Molly Plant Food is said to provide the same euphoric feeling as Ecstasy. This synthetic drug is said to contain either methylone (3, 4-methylenedioxy-N-methylcathinone), MDPV, mephedrone (4-methylmethcathinone), or many other methcathinones derivatives.[2] Many users of this fake Ecstasy do not know that it closely resembles methamphetamine, lacking only two functional groups. The primary buyers of these drugs are teens that may not be aware of the dangers of this drug. In recent years, there have been many cases of hospitalization and even death resulting from the use these drugs. As previously mentioned, the effect of this drug resembles that of Ecstasy; however, other compounds are added to mask its intended use as a drug of abuse and to allow it to be advertised as a plant growth agent or muscle relaxant. Since not much is known of this drug, it has the capacity to be abused at high levels, which can lead to death.

The research used high performance liquid chromatography to retrieve the different kinds of chemical components present in the Molly Plant Food.[3] The results of the LC/MS showed the presence of mephedrone and MDPV in the Molly Plant Food. Mephedrone and MDPV are just two of the many methcathinones found in various illegal synthetic stimulant drugs. The use of Raman spectroscopy provided a spectrum of the chemical constituent. The Raman findings showed strong bands at 2950cm^{-1}, 1750 cm^{-1}, 1020 cm^{-1}, and 770cm^{-1}. These findings provide a general distinctive identifying characteristic for the illegal compound. The finding of exact transition ion intensities at 10V, 27V, 36V, and the existence of methcathinone were confirmed via LC/MS. Further, the pyrolysis-GC/MS conducted on Molly Plant Food showed a high concentration of analgesic chemicals of the naphthoylindole family. The use of high temperature nickel-cobalt foil at 590°C and 670°C showed the presence of JWH-018 and JWH-073 when Molly Plant Food was pyrolyzed. These compounds were recently used in many synthetic cannabis products to give the same "high" effects of those of THC.[4] Since the drug usually comes in capsule or powder form, the understanding of the chemical constituents of these drugs makes makes them easier to identify.

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Toxicology, Stimulant Drug, Methcathinone

A27 Variability in a Mossberg Model 500 Shotgun's Firing Pin and Breech Face Impressions

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After attending this presentation, attendees will understand how firing pin impressions correlate back to a specific shotgun, and how this information helps fill the gap between the uniqueness of shotgun evidence and its interpretation.

This presentation will impact the forensic science community by providing statistical data that helps support the reliability and use of the IBIS within the justice system.

The purpose of this study is to examine the precision (inter-day variability), repeatability (intra-day variability), reliability (consistency and stability of results), and correlations (degree to which variables are related as opposed to causality) between firing pin and breech face impressions in shotgun evidence.[5]

There is much controversy related to firearms evidence, specifically, in analyzing the relationship between crime scene evidence and evidence found in the possession of a suspect. Minimal research exists investigating the value of this evidence. Ogihara *et al*., (1983) and Shem & Striupait (1983) performed comparisons of 5001 and 501 consecutively fired bullets with .45 and .25 caliber semi-automatic handguns, respectively, by firing pin impressions.[1,2] Grove, Judd, & Horn (1972) also examined firing pin impressions using scanning electron microscopy.[3]

The research used the Integrated Ballistics Identification System and a Leica FS-C comparison macroscope. The IBIS system uses bullets and casings from case evidence from a crime scene and compares them to a database of known fired weapons. IBIS consists of a fully automated projectile and cartridge case comparison systems, BULLETPROOF® and BRASSCATCHER™, respectively.[4] These systems allow the IBIS examiner to compare across a known database within minutes. IBIS provides a relative score for each comparison, and a list of highest matching breech face and firing pin scores is generated that represents the highest matching comparisons in the database.[4]

The Leica FS-C comparison macroscope was used to test the reliability of the IBIS. The reliability was tested by using best known non-matches when present. The macroscope compared those to other casings that have been integrated into the IBIS and known to have been fired using the same firing pin. The FS-C was also used to compare the firing pins against corresponding firing pin impressions.

A Mossberg Model 500 twelve-gauge shotgun was used to perform all test firings. Remington ammunition (2¾ Express,) was used while varying the pellet size (00, #4, #6, and #8 (all lead). The firing pin in the shotgun had an unknown history. The firing pin scores generated with this firing pin were used as a baseline. All tests were repeated with four new firing pins.

The test fires were conducted at an outdoor shooting range and each shotshell was collected directly after firing. Upon logging the shotshells, they were then entered in the IBIS system and the correlation worksheets were generated.[4] The breech face and firing pin scores were analyzed using Microsoft Excel™. The effects of shot type on firing pin scores were evaluated. The intra-variability and inter-variability of firing pin scores relative to individual firing pins were contrasted. Receiver Operating Characteristic curves demonstrating the ability of using the firing pin score to effect identification was constructed. Since the breech face impression of all shots fired remained constant, it provided a basis for evaluating the intra-variability of breech face scores, and allowed for the examination of cartridge cases in firearms where the firing pin has been modified or replaced.

The data was evaluated to test the following hypotheses: (1) there is a higher correlation between the same load sizes shot from the same firearm (or the intra-variability of different load sizes fired by the same firearm is smaller than the inter-variability of varying load sizes); and, (2) fired ammunition will have higher breech face and firing pin scores when comparing intra-firearm scores and inter-firearm scores.

The data was exported into Netica™, a program for designing decision diagrams. A Bayesian network was created using the data to represent the variability relationships graphically. The output was a likelihood ratio estimating the probability of the prosecutorial hypotheses relative to the probability of the defensive hypotheses thus providing an indication of the weight of the evidence.

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Firing Pin, IBIS, Mossberg Model 500

A28 Second and Third Generation Legal Highs

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After attending this presentation, attendees will have understanding of the emergence of second- and third-generation legal highs.

This presentation will impact the forensic science community by presenting a selection of the emerging drugs of abuse that drug chemists may encounter in casework in the near future.

On July 9, 2012, President Obama signed the Synthetic Drug Abuse Prevention Act of 2012, intended to control chemicals designed to mimic marijuana and amphetamines. On July 12, 2012, "*Wired Science*" declared the ban obsolete. The legislation was in response to two emerging drugs of abuse: spice and bath salts. When the public first became aware of these drugs in 2008, bath salts were primarily the cathinone analogs, methylone, mephedrone, and methylenedioxypyrovalerone (MDPV). Spice, sold as synthetic marijuana, was not a cannabinoid at all. The psychoactive substances in spice were cannabinoid agonists, chemicals that bound to the CB1 and CB2 cannabinoid receptors. The most prevalent were the JWHs developed by John W. Huffman in the 1990s. A few examples are JWH-018, JWH-073, JWH-019, JWH-200, JWH-250, JWH-081, WH-122, and JWH-398.

Individual states were the first to respond. By late 2011, over 30 states had banned the cathinones and several of the spice ingredients. The online vendors immediately responded with new "legal highs." Some even had state-specific websites. The most prevalent second-generation legal highs were naphyrone, butylone, and 5, 6-methylenedioxy-2-aminoindane (MDAI). The cannabimimetics were extended to include C8 homologs, AM2201, AM678, and many more JWHs.

As quickly as one class of legal highs is banned, new drugs are put on the market. They are sold online, in gas stations, and in head shops. They are often disguised as "bath salts" or "plant food." These substances are of concern because numerous incidents of overdose, organ damage, and even death have resulted from their consumption. The drugs are untested and unregulated. Distributors have no quality assurance and there is no guarantee that a person is receiving the drug they intended to purchase.

The objective of this project is to purchase new legal highs as they are offered online and in local head shops. The information found in this study is disseminated to poison control centers and lawmakers to help in treatment of patients by health professionals, to warn the public about the dangers of these substances, and to aid policy makers in preventing their distribution.

The samples are generally in the form of a powder or plant material. Each sample is ground in a mortar and pestle to homogenize the material and ensure a representative sample. The powders are extracted by two methods; an acid/base and extraction directly into hexane. Plant materials undergo an additional sonication step in both the acid extraction step and the direct hexane extraction. The samples are analyzed by gas chromatography and mass spectroscopy. If identification cannot be determined by the mass spectra, ESI and NMR are used to determine the structure.

In this study, several legal highs were analyzed for active components. The legal high, "synthacaine" was ordered online and found to contain a mixture of methiopropamine (MPA) and benzocaine. MPA is a thiophene-based analog of methamphetamine. Another drug, sold contains a mixture of MPA and MDAI. MDAI is a drug that can produce effects similar to MDMA. Other substances analyzed include legal highs containing 6-APB, of wich all contained the second-generation canniabimimetics, UR-144, XLR-11, A-796,260.

Legal Highs, Cannabimimetics, Cathinones

A29 Effect of Organic Modifiers on Separation of Fluorescently Labeled Phenethylamines in Capillary Electrophoresis

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After attending this presentation, attendees will gain better insight into the role of organic modifiers in electrophoretic separations of fluorescently-labeled phenethylamines. The relationships between concentration of the fluorescent tag, 5-(4,6-dichloro-s-triazin-2-ylamino)fluorescein (5-DTAF), labeling efficiency, and peak resolution will be discussed.

This presentation will impact the forensic science community by detailing the optimization parameters of a potentially excellent screening method for trace amounts of phenethylamines and other related compounds, which are highly efficacious at low doses.

In electrophoresis, electric potential is applied at the ends of a capillary filled with an electrolytic solution resulting in the generation of an electric field. This field causes the movement of the electrolyte, causing any analytes present to be separated based on their mass-to-charge ratios. Due to the small inner diameter of these capillaries, sample and reagent volumes used are in the nanoliter range making it very cost-effective. Due to the short optical path length required for

capillary columns, a commonly utilized detection method for this separation technique is laser-induced fluorescence. This is because of its high sensitivity and specificity, which allows for the detection of compounds in the ng/mL range. For this study, five commonly encountered drugs and precursors (used in illicit preparations) were investigated: amphetamine, methamphetamine, norephedrine, ephedrine, and methylenedioxyamphetamine (MDMA). Since fluorescence is not a property native to any of these analytes, they must first be coupled to a fluorescent molecule, in this case 5-DTAF, in order to make them compatible with the technique. This process is known as fluorescence derivatization.

Given their small size and overall similarity in structure, the phenethylamines investigated all have similar pKa values and migration rates. Due to this, coelution of peaks is a commonly encountered difficulty. To overcome this and improve the separation between the individual drugs assessed, modifiers were added to the run buffer to alter the electro-osmotic flow and thus the velocity of the bulk solution as well as vary the migration rates of the analytes through their interactions with them. These modifiers included various β-cyclodextrins and organic solvents. As a result, the individual drugs separate into distinct zones. As they pass through the detection window on the way to the cathode, samples are irradiated by the laser. The signal produced from this excitation and subsequent emission is then collected by the detector and converted into an electropherogram for interpretation.

Drug standards were dissolved in analytical-reagent grade methanol for storage at 4°C. Prior to analysis, samples were diluted to appropriate concentrations using deionized water from a ultrapure water purification system and methanol. For this method, a micellar running buffer comprised of 50mM borate, pH 9.5/15mM β-cyclodextrin (β-CD) was used for the separation of the analytes. A background electrolyte of 50mM borate, pH 9.5 and a derivatization buffer of 0.5M $NaHCO_3/Na_2CO_3$, pH 9.5 were also utilized. Experiments were conducted using a CE-based analytical system unit interfaced with a computer. The fused-silica capillary was 60.5cm (effective length 50cm) with an internal diameter of 50µm. An argon ion laser was used as an excitation source (488 nm) and electropherograms were recorded by monitoring the emission intensity at 520nm. New capillaries were conditioned by thorough rinsing with 0.1M sodium hydroxide, deionized water, and micellar running buffer in series.

Phenethylamines, Electrophoresis, 5-DTAF

A30 A Step Toward the Development of Methods for the Analysis of Fingerprint DNA

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The goals of this presentation are to provide information about the components of a deposited fingerprint sample, and to discuss a technique for the generation of a positive control with analysis of the results.

This presentation will impact the forensic science community by providing basic information about the components of a touch DNA sample originating from a fingerprint, while also discussing and presenting methods for DNA analysis using the standard, widespread acceptance of such techniques.

"Touch DNA" is contained in the cells deposited by a person's physical contact with an object such as a hard surface, that is, the deposition of a fingerprint. Studies have shown that is it possible to amplify both mitochondrial and nuclear DNA from fingerprints, and this could provide valuable evidence in many circumstances. However, the biological origin of the DNA in fingerprints is not completely clear. It may arise from sources such as epithelial cells trapped in skin oils or from cell-free DNA found in body fluids. An elucidation of these mechanisms could prove invaluable in the forensic analysis of samples.

The goal of the project presented here was to study basic characteristics of deposited fingerprints to aid in the development of techniques for the collection and analysis of this touch DNA. The first step was the generation of a suitable positive control for use in the experiments. A quantifiable, non-variable sample containing DNA and other components that mimicked a fingerprint (i.e., deposited cells in a chemical matrix) was required. A simple, cost-effective technique was developed for the production of these controls. Briefly, the most abundant chemical components comprising a fingerprint were combined proportionally to make the "fingerprint solution." Buccal epithelial cells were collected and suspended in the fingerprint solution. They were treated to reduce clumping and counted in a hemocytometer. The number of cells was equated to the DNA content of the solution, which allowed a known quantity of DNA to be used as a positive control while retaining the chemical characteristics of a fingerprint.

The cell/fingerprint suspension was used two ways: (1) it was deposited on a hard surface and spread with the use of a small roller to mimic a fingerprint; and, (2) it was added directly to an extraction reaction to allow for an estimation of the number of cells lost during the deposition procedure. Early in the project, these control samples consistently showed extraction yields greater than 100 percent, indicating the need to more carefully examine the components of a body fluid extract, especially when it contains limited template DNA. In subsequent studies, the source of the DNA in each type of sample was considered. Origins of the DNA such as endogenous extracellular sources, contamination from various collection methods, and contamination introduced during the procedure were examined. The results will be discussed here and the procedures for the generation of a positive control will be presented.

Fingerprints, DNA, DNA Profiling

A31 Forensic Analysis of Carpet Fiber Samples Using Direct Analysis in Real Time Coupled to an Accurate Time-of-Flight Mass Spectrometer

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After attending this presentation, attendees will understand how the analysis of carpet fiber samples in forensic laboratories involve a series of different tests, including the comparing of fibers microscopically, through the use of micro-chemical and micro-solubility tests, FTIR microscopy, etc. This study will attempt to test the ability of the DART®-AccuTOF™ for fiber analysis in either a screening or confirmatory capacity.

This presentation will impact the forensic science community by demonstraiting how DART®-AccuTOF™ was able to correctly identify and distinguish the various polymer classes and sub-classes (i.e.; Nylon versus Polyester as well as Nylon 6 versus Nylon 6/6). Although it is destructive, the rapidity of the technique along with its high power of discrimination makes it a favorable test to be either added to the series of tests, or can even replace a few of them.

Fibers are associative evidence that is encountered in numerous forensic circumstances. They can be found in crime scenes that involve breaking and entering, hit and runs, and even rape. This study focused on nylons, polyesters, and olefins, which are the most frequently encountered carpet fibers. An attempt to analyze the multiple polymer types was done using Direct Analysis in Real Time (DART®) coupled with accurate mass spectrometry (AccuTOF™). Twelve nylon, polyester, and olefin polymer standards were used to optimize parameters for the analysis of carpet fibers. A DART® helium gas temperature of 275°C was chosen as an optimum for analysis due to the differences in melting point for the various polymer types. Use of collision-induced dissociation to enhance fragmentation increased the power of discrimination, albeit decreasing sensitivity. A function switching method between 20 and 30 volts was established in order to provide greater spectral detail, while

minimizing fragmentation which would lead to an even more cluttered spectrum. Minimal sample preparation was needed to analyze the samples; however, due to the small sample gap between the DART® ceramic and orifice, care was taken in order to not obstruct the orifice with the samples. This involved sampling the carpet fibers using a pair of tweezers, which was taped to a dowel, held tightly with a clamp. The main disadvantage of this process is that it is a destructive technique. All 12 polymer standards were successfully differentiated and identified using DART®-AccuTOF™ based on the presence of their monomers and associated dimers and trimers. A total of 32 carpet samples were analyzed in this study. Carpet samples of known compositions were correctly identified by class, and the remaining carpet samples of unknown compositions were also correctly identified following FTIR identification. In addition to identifying polymer class, the sub-class was identified in some cases such as nylon 6 versus nylon 6/6. All carpet fiber spectra demonstrated an intense peak at 282 Da, which was attributed to oleamide, a slipping agent that is used to enhance the extrusion of the fiber strands through the spinneret in the fiber-making process. The results demonstrate the capability of DART®-AccuTOF™ being implemented as an addition to the series of tests conducted to analyze carpet fibers. In addition to carpet fibers, other types of fabric and materials may also be analyzed using this technique. Reproducibility studies may allow for the use of this technique to directly compare known and questioned fiber evidence.

DART® AccuTOF™, Carpet Fibers, Nylon

A32 Searching for the Elusive Spermatozoa: Revisiting Seminal Fluid

Kelsey R. Sakaida, Kyle A. Williston, Kimberly A. Strong, and Reena Roy, PhD, Penn State Univ, Forensic Science Program, 107 Whitmore Laboratory, University Park, PA 16802*

After attending this presentation, attendees will understand how to find rare human spermatozoa on a low budget without resorting to expensive equipment and software.

This presentation will impact the forensic science community by teaching the practicing biologist how to detect human spermatozoa when they are extremely rare in a specimen and also mixed with spermatozoa of other animals and other human body fluids.

Forensic crime laboratories analyze evidence from various types of sexual assault cases. The most likely source of male DNA in sexual assault cases comes from semen, which contains male cells commonly known as spermatozoa. Although most of the sexual assault cases involve humans, forensic bestiality cases are also common, and differentiating human male cells from animal spermatozoa becomes a necessity. In such cases, it is important to confirm the presence of human spermatozoa for the investigators and attorneys to bring charges against an assailant.

Examining such evidence to identify spermatozoa requires a great deal of time and effort using stains such as Kernechtrot-Picroindigocarmine (KPIC) staining, commonly used in the crime laboratories for the visualization of sperm. In some instances, because of the rarity of the spermatozoa in the evidence, an analyst may not be able to detect the KPIC-stained heads of the spermatozoa.

The goal of this research was to manually identify human spermatozoa where mixtures of human and animal spermatozoa exist. Some of the simulated evidence samples contained body fluids such as blood and saliva as well as seminal fluids from humans and animals. Another objective was to detect rare human spermatozoa from slides that have been prepared from vaginal swabs collected several days after coitus and where the cells have been dyed with dyes commonly used in the crime laboratories. The project included re-staining these KPIC-stained slides with immunofluorescent dyes to enhance the detection of the male cells.

SPERM HY-LITER™ technology in conjunction with fluorescent microscopy and specific computer software to detect spermatozoa is a novel method in the forensic community. This immunofluorescence staining technique is specific for human sperm cells since it does not stain animal spermatozoa, human epithelial cells, or other types of body cells that may be present in the sample. In some instances, where the victim does not report the crime for several days and spermatozoa may become rare and difficult to detect, this immunofluorescent technology can detect rare spermatozoa among many other non-human spermatozoa and human cells. This technique also allows the detection of rare spermatozoa present as one of the components in a complex mixture of other body fluids. The microcopy and the software to search for the stained spermatozoa are expensive, and budgetary constraints may not allow a laboratory to use such tools.

In this research, SPERM HY-LITER™ was used to stain human and animal spermatozoa. Once stained, these cells were analyzed manually by a fluorescent microscope which did not have the expensive computer software necessary for the automatic detection of stained human spermatozoa. The goal of this research was to identify human spermatozoa where a mixture of human and animal spermatozoa may exist. Another objective was to be able to detect rare spermatozoa even after obtaining vaginal swabs several hours after coitus. In one of the aspects of the study, slides were prepared from vaginal swabs and stained with KPIC. Once the spermatozoa were visualized, the cells were re-stained with fluorescent dye. In all of the instances, human spermatozoa were identified from various samples analyzed by this method.

The results obtained from this research would benefit the other forensic biology laboratories as they can use this technology accurately and efficiently for identification of spermatozoa without straining the budget.

Spermatozoa, Animal Semen, Human Body Fluid

A33 A Comparison of Mitochondrial DNA Species Identification Techniques of Questioned Animal Samples

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After attending this presentation, attendees will understand several PCR-based methods available for determining the origin species of biological material and the advantages and disadvantages of each.

This presentation will impact the forensic science community by introducing a variety of established DNA-based methods that are used to identify the species of origin of tissues or other biological materials and describing extensive experiments for their comparison regarding successful PCR amplification, sequencing, and identification of their species of origin.

It is not uncommon for legal authorities or forensic practitioners to be confronted with biological material of unknown origin. From these, two questions arise: (1) “Is the material human?”; or, (2) “What is the species of origin?” The former can be key in missing persons cases when a search turns up biological material, while the latter is important when material clearly animal in origin, but otherwise undefined, is encountered.

Modern DNA methodologies have allowed molecular biologists to identify regions of the animal genome that are similar enough among species such that they can be successfully PCR amplified using universal primers, while still varying enough between primers so that a more precise origin can be determined, be it species, genus, etc. The region between primers is sequenced, and the resultant sequence undergoes a query using Basic Local Alignment Search Tool (BLAST: NIH’s on-line database of DNA sequences). This database has so much information available that a clean sequence virtually always results in matches in the database that are specific to a species. In this way, the species of origin of biological material is confirmed.

Multiple laboratories have developed and published protocols for molecular species identification. Virtually all are based on mitochondrial DNA loci that readily fit the conserved primer/variable internal region criteria needed for the assay. The first of these came from Alan Wilson’s

molecular evolution laboratory at UC Berkeley, and many others have followed, including the Michigan State University laboratory. MtDNA loci utilized the range from the most highly conserved mtDNA genes to the least conserved loci. Each of these species identification loci has its potential advantages and drawbacks, including overall universality, ease of amplification, ease of sequencing, and specificity.

In the current study, all mtDNA-based methods for animal species identification available, including those used in house, were compared based on the above criteria. The list of loci examined, their numerical location based on the human mtDNA reference sequence, and amplicon length, are displayed in Table 1.

Primer	Region	Base Pair Length
12S	1071-1199	128
16S 1	2489-2716	227
16S 2	2676-3007	331
Cytochrome Oxidase I	5926-6609	683
Cytochrome b 1	14724-15915	1191
Cytochrome b 2	14816-15173	357
Control Region 1	15995-16498	503
Control Region 2	15908-16498	590
Control Region 3	15735-16498	763

Sixty-six species were examined, consisting of 27 mammals, ten fish, seven birds, five reptiles, three amphibians, nine insects, one centipede, one millipede, one spider, one earthworm, and one crustacean. Amplification was attempted on each based on the loci listed in Table 1, and graded as either positive, weak positive, or negative. From there, a subset of species that produced amplicons, consisting of one white tail deer, bobcat, lake white fish, frog, bearded dragon, English sparrow, house centipede, click beetle, pill bug, and earthworm, was reamplified and the same primers were used for forward and reverse sequencing. Sequences were aligned and edited, and a BLAST search was conducted. The sequences were then scored for BLAST accuracy (correct species identified) and uniqueness (sequence differed from other species).

The most useful loci in regards to amplification of the widest range of species included 12S, 16S 1 and 2, and cytochrome oxidase I, all of which amplified in a large majority of species. In contrast, cytochrome b 1 faired poorly overall, most likely due to the large size of the amplicon. Of the three control region loci, CR 3 amplified in the most diverse set of species, while the other two were effective in mammals and birds, but less so in the remainder.

The best generated sequence data overall was 16S 1 and cytochrome b2. BLAST search results of quality sequences were consistent with the known species, although the small size of the 12S and 16S 1 amplicons often resulted in complete matches with related organisms, thus precise species identification was not possible. In other instances, it is likely that the explicit species, or locus from that species, was not in the database at all. For instance, there are over 900 species of click beetle in North America, yet only one showed up in the database for 16S 1, and none for cytochrome b 2, indicating the specific species studied was not represented. This is a clear limitation of the technique in general; however, species most likely to be investigated by forensic scientists tend to be well represented in the database. Overall, utilizing mtDNA for species identification is an effective and widely applicable tool, while the locus, primers, and amplicon sizes utilized require careful consideration.

Species ID, Mitochondrial DNA, DNA Species ID

A34 Identification of Body Fluid Traces Using Raman Spectroscopy: Toward Practical Application

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The goal of this presentation is to describe the development of a novel method for non-destructive, confirmatory identification of body fluid traces on the crime scene and in the laboratory. Attention will be focused on the most recent development of the method for characterization of contaminated samples, mixtures of body fluids, and possible substrate interferences. The attendees will also have a better understanding of the recent advancement of this application of Raman spectroscopy. The implementation of advanced statistics for automatic 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 offering the potential to greatly impact the accuracy and effectiveness of biological stain analysis for forensic purposes.

The identification of traces of body fluids discovered at a crime scene is a major part of forensic investigation today.[1] The three most common fluids found are blood, semen, and saliva, and there are several methods used currently to distinguish one from another. Blood can be presumptively tested by using different color spot tests, but these tests are destructive to the sample and can also produce false positives. Semen is similar in that there are destructive presumptive tests as well as confirmatory tests. Saliva; however, has no confirmatory tests. Most presumptive tests can be performed in the field, but some sample preparation such as extraction is often necessary. Most confirmatory tests must be done in the laboratory. The main problem with these tests is the destruction of the sample. The forensic science community is in great need of a reliable, non-destructive, on-field method for identification of all common body fluids.

Raman spectroscopy is a technique that is increasing in popularity among the different disciplines of forensic science. Some examples of its use today involve the identification of drugs, lipsticks, and fibers, as well as paint and ink analysis. 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 in the field and still be available for further use in the laboratory for DNA analysis. A portable Raman spectrometer is a reality now that should allow for use at the crime scene.

Reported here is the latest development of a new method for identification of body fluid traces using Raman spectroscopy combined with advanced statistics. Multidimensional Raman spectroscopic signatures of dry traces of sweat and vaginal fluid were developed in addition to the signatures of semen, saliva, and blood reported earlier.[2-4] Combined software was developed for the identification of all major body fluids and the evaluation of the accuracy and reliability of the conclusions made. The method was expanded for the application to blood and semen samples contaminated heavily with sand, dust, and soil.[5] The ability of the method to detect and identify small amounts of semen and blood in their mixed samples will be reported.[6] Potential interferences from common substrates will be discussed.

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Raman Spectroscopy, Body Fluid, Statistics

A35 Optimized Centrifugal Methods for Separation of Semen From Superabsorbent Polymers for Forensic Analysis

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After attending this presentation, attendees will gain an understanding of the challenges in separating cellular material from the Superabsorbent Polymer (SAP) materials and the fibrous matrices found in evidence such as diapers, sanitary napkins, absorbent medical pads, and other related forensic evidence, and a protocol that can successfully separate the cellular material from the substrate.

This presentation will impact the forensic science community by describing best practices for isolating semen from evidence containing absorbent and superabsorbent materials, and the impact that SAPs has on downstream DNA analysis.

The best evidence to connect a perpetrator to a sexual assault is the confirmed presence of semen, which through forensic examination can prove sexual contact by verifying ejaculation and/or penetration. Sexual assault cases can involve evidentiary items such as sanitary napkins or diapers, which contain superabsorbent polymers (SAPs). SAPs are cross-linked polymeric materials that can absorb and maintain large amounts of aqueous solutions without compromising structure. Ejaculation of semen onto these SAP-containing substrates results in cellular material, including spermatozoa, becoming entangled in the SAP gel-like mesh. Thus, separation is difficult and the likelihood of a typable spermatozoa yield is questionable. Additionally, visualization and proper identification of spermatozoa through microscopic examination can become problematic when SAPs are present; therefore, common practice involves working with only the top cotton or paper layers of these types of evidence to avoid SAPs whenever possible.

The success rate of obtaining DNA profiles from SAP-containing evidence has not been evaluated in a systematic fashion. It has historically been assumed that residual SAPs in the purified DNA extract can interfere with and create complications for downstream forensic applications. Therefore, an evaluation on the effects of SAPs on forensic DNA processes was necessary, as was the development of a simple, efficient protocol for separating cellular material from evidence containing SAPs. A number of centrifugal-filtration methods were evaluated to determine best practices for isolating biological materials from SAP-containing evidence. In order to select the most effective filtration device, excisions of commonly used sanitary napkins, diapers, and adult incontinence products containing 100 µL depositions of 1:5 human semen were filtered utilizing five different types of centrifugal filters. The selection of the best filter was based on spermatozoa yield, DNA yield, and ease of use. The resulting filtrates were microscopically examined for presence of spermatozoa and percent yields were calculated and compared. DNA from each sample type was isolated using a differential organic extraction method and was evaluated for DNA yield and quality. Shifts in Internal Positive Control (IPC) cycle thresholds and melt curves were assessed to address possible inhibition caused by the presence of SAPs. Results indicate that polyester fabric-layered basket filters were simplest to use and resulted in significantly higher spermatozoa and DNA yields than other centrifugal filter methods. No indication of significant inhibition by residual SAPs was observed in any of the filtered DNA extracts.

A sampling comparison was made between the top cotton or paper layer of the SAP-containing substrates versus taking the entire substrate excision. This set of experiments sought to determine the best sampling method as determined by spermatozoa visualization and DNA yield. Results indicated that sampling the entire excision of the SAP-containing substrates yielded a significantly higher quantity of visualized spermatozoa and DNA yield than just sampling the top layer of the evidence. As no PCR inhibition from the SAPs was observed in the entire excision samples, it is therefore recommended that the full depth of SAP-containing evidence be sampled for spermatozoa identification and DNA analysis in forensic casework.

In conclusion, filtering samples taken from evidence containing SAPs greatly improves the screening process of spermatozoa identification and makes these samples easier to work with for downstream processes. The optimized filtration method also allows for subsequent separation of the sperm and non-sperm fractions, and processing of biological samples using a variety of validated forensic DNA isolation protocols. There is a significant increase in sperm and DNA yield when the entire excision is filtered in comparison to the common practice technique of taking only the top layer in order to avoid the SAP gel portion. The yields of spermatozoa and DNA purified from these filtered samples show promise that usable STR profiles can be obtained.

Seminal Fluid, Absorbent Polymers, Spermatozoa

A36 Evaluation of DNA Extraction Efficiency

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After attending this presentation, attendees will understand the importance of evaluating extraction efficiency from a known amount of DNA, learning that the observed recovery value range was significantly lower (20 to 30 percent) than many reported extraction efficiency calculations using the number of full STR profiles produced.

This presentation will impact the forensic science community by bringing attention to the amount of DNA unrecovered during the extraction process. The evaluation of the amount of unrecovered DNA could lead to more efficient methods to recover higher percentages of DNA from the extraction and purification processes.

Forensic DNA typing requires a specific quantity of input DNA (typically 0.5 – 1.0 nanograms) to generate an optimal Short Tandem Repeat (STR) profile. For reference samples, the amount of DNA collected on a standard buccal swab or blood punch is generally in excess of that which is needed for testing (on the order of hundreds of nanograms (ng)). Typically, extraction efficiency is evaluated by determining the number of samples that produce a full STR profile divided by the total number of samples processed. Less attention has been paid to the amount of DNA unrecovered during the extraction process from the original sample. Determining the amount of unrecovered DNA after the extraction process requires the original amount of DNA to be known prior to extraction, which is not the case in reference or casework samples. The importance of evaluating the theoretical yield versus the functional yield is in cases when the initial amount of available DNA is low. Within the extraction process, a majority of the DNA sample is lost, which has minimal impact on reference samples because enough DNA is recovered for an STR profile, but can have a significant impact non-reference samples within a laboratory. In these cases, it would be beneficial to obtain an extraction recovery that is closer to the theoretical yield than the functional yield. Evaluating the amount of unrecovered DNA could lead to more efficient extraction methods to recover higher percentages of DNA from the extraction and purification processes.

Extraction efficiency experiments were conducted to evaluate the percentage of DNA recovered through two extraction methods: a salting out procedure and use of the Qiagen EZ1 Advanced XL extraction robot with the DNA Investigator kit.[1] Three DNA sources were tested using varying initial amounts of human cells, previously purified and highly characterized DNA, and liquid whole blood. Controlled amounts of DNA from the three DNA sources were absorbed onto cotton buccal swabs, specimen collection paper, and FTA paper. The cells were spotted onto the swabs and paper in a PCR-compatible buffer suspension consisting of a 1.0 % BSA, 0.9 % NaCl, and 10 mM TRIS solution. Theoretical DNA quantities were estimated in total nanograms of DNA and applied to estimate a recovery percentage for each extraction. Human cells were quantified using a Coulter Counter and suspended within the PCR compatible buffer for each of the appropriate concentrations. The white blood cell count of a healthy individual ranges between 3.5 million and 10.5 million cells per milliliter, and a value of 7.0 million white blood cells per milliliter was used to determine theoretical DNA quantity for all whole blood samples.[2] The theoretical DNA series examined ranged from 24ng to 1800ng for all sample types. All extracted samples were quantified with Life Technologies Quantifiler Human DNA quantification kit in replicates of two. Results indicated that extraction efficiency ranged from 20% to 30% and recovery was independent of extraction method and DNA source. The observed recovery value range was significantly lower than many reported extraction efficiency calculations relying solely on the number of full STR profiles obtained.

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Extraction Efficiency, DNA, Forensic DNA Typing

A37 DNA Isolation and Analysis From Skeletal Remains: Novel Methods for Removing PCR Inhibitors

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After attending this presentation, attendees will appreciate common difficulties encountered during DNA analysis of skeletal remains and understand the extent to which soil microbial DNA isolation kits can act as an advantageous extraction method for buried skeletal remains. Attendees will also be informed about which DNA extraction methods are most useful for recovering amplifiable DNA from buried human skeletal remains and removing Polymerase Chain Reaction (PCR) inhibitors.

This presentation will impact the forensic science community by disseminating whether microbial DNA isolation kits are more effective at purifying DNA away from PCR inhibitors than are commonly used DNA isolation methods, as these kits have never been assessed for their effectiveness in extracting clean DNA from skeletal remains.

DNA analysis of skeletal remains is crucial in order to identify missing persons, victims of war, and individuals in cases of mass disaster. Unlike reliable sources of DNA such as buccal swabs, challenges arise with skeletal DNA analysis for numerous reasons. The harsh conditions skeletal remains are often recovered from are not conducive to DNA preservation, such as wet environments or the heat of a fire. The presence of PCR inhibitors is also a common hindrance with skeletal remains, particularly if they have been buried or are otherwise in prolonged contact with soil. Likewise, components of the bone itself, such as calcium or collagen, can inhibit PCR, and thus the removal of PCR inhibitors during DNA extraction is critical for successful forensic DNA analysis.

Previous researchers have compared DNA extraction methods from skeletal material, including standard phenol-chloroform organic extraction and commercially available kits. In these studies, the kits had low DNA recovery, instances of PCR inhibition, and resulted in poor quality STR profiles.[1,2] Organic extraction recovered higher quantities of DNA; however, this method also resulted in PCR inhibition.[2] Although commercial DNA isolation kits are easy to use and claim to remove PCR inhibitors, none have been optimized for the highly compromised skeletal remains that are often encountered by forensic scientists, which is presumably why they have been found lacking for skeletal analyses. This led to the question of whether commercially available kits that are specifically designed to isolate and purify DNA from soil samples that are high in PCR inhibitors such as humic and fulvic acids might be advantageous when testing buried skeletal remains.

In the research presented here, the ability of microbial DNA isolation kits to recover amplifiable bone DNA and remove PCR inhibitors was compared with other common extraction methods. DNA extraction systems included a PowerSoil® DNA Isolation Kit (MoBio), a SoilMaster™ DNA Extraction Kit (EpiCentre), a standard organic extraction, and a QIAamp® DNA Investigator kit (QIAGEN), which has a specific protocol for DNA isolation from bone. Since the soil kits are not designed for extraction of human materials, a preliminary study was conducted to determine if reagents contained in the kits were contaminated with human DNA. Blank extracts from the soil DNA kits failed to amplify with human mtDNA primers. Next, DNA extractions were performed on bone powder obtained from drilling cow femur segments to determine whether the standard protocols supplied with each are adequate for DNA isolation from bone. The PowerSoil® kit standard protocol did not consistently result in amplifiable DNA. The protocol was then optimized by altering the mechanical/chemical digestion step, including substituting a hot lysis for the mechanical digestion.

In addition to DNA recovery, each extraction method was tested for its ability to remove the PCR inhibitors calcium, collagen, and humic acid, which are associated with buried skeletal remains. Inhibitor removal was assessed by amplification or no amplification of control mitochondrial DNA, by adding the purified extract to the reaction. Each extraction method was then used on bone powder produced by drilling cow femur sections buried in soil for a range of time: one day, seveb days, thirty days, and three years. DNA was quantified by a real-time PCR TaqMan assay targeting the Melanocortin-1 Receptor gene, developed by Lindquist *et al*.[3] Inhibition was assessed by comparing cycle threshold values of the internal positive control. Since failure to amplify DNA is a common challenge encountered with skeletal remains, successful amplification of both mitochondrial and nuclear DNA was compared for each extraction method to see which recovered amplifiable DNA more often. The four extraction methods were then tested on various human skeletal remains, including bones from the medieval period, which had previously shown PCR inhibition during DNA analysis. After comparing the microbial DNA isolation kits to organic extraction and a standard DNA extraction kit for inhibitor removal, quantity of bone DNA recovered, and success of mitochondrial and nuclear DNA amplification, the effectiveness of microbial DNA extraction kits for use on skeletal remains was determined, as well as the most optimal extraction method.

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DNA, Skeletal Remains, PCR Inhibition

A38 Girls Not Allowed: Erase the Mixture

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After attending this presentation, attendees will learn how to separate male and female DNA fractions in sexual assault cases without going through the cumbersome differential extraction procedure.

This presentation will impact the forensic science community by discussing a procedure that can be used for identifying the semen donor and separate the DNA of the male donor from the epithelial cells of the victim.

Forensic science laboratories process numerous cases involving sexual assaults. When the victim goes to a health care facility, a sexual assault kit is used to collect body fluids from various orifices. The swabs used to collect fluids from vaginal, rectal, and oral cavities often contain a mixture of the victim's epithelial cells and seminal fluid from the suspect. If the victim does not report the crime within a few hours, the number of epithelial cells from the victim can overwhelm the number of sperm cells present in the sample collected from the victim. Normally, a time-consuming and cumbersome differential extraction procedure is used in an attempt to separate the sperm DNA from the epithelial DNA. The resulting sperm DNA profile may yield a mixture of sperm and epithelial cell DNA even after repeated differential extraction wash steps.

The Erase Sperm Isolation Kit (Paternity Testing Corporation) can degrade DNA from epithelial cells, while leaving the spermatozoa intact. The DNA from the epithelial cells is selectively degraded by nuclease capable of digesting the DNA that is in solution. The sperm cell membrane protects the DNA inside the sperm heads and the nuclease has little effect in the protected DNA. This process makes it possible to obtain a single source sperm cell autosomal DNA profile from samples that also contain overwhelming amounts of epithelial cell DNA. Therefore, it is possible to obtain a single source male DNA profile from a mixture of seminal and vaginal fluid using the reagents in the kit.

The goal of this study was to determine if the Erase Sperm Isolation Kit could separate sperm cell and epithelial cell DNA from post-coital swabs, and from samples containing mixtures of body fluids. The objective was to generate single source male and female DNA profiles from various mixtures containing female body fluids and seminal fluid containing spermatozoa.

Swabs containing a mixture of seminal fluid and vaginal epithelial cells were collected at regular intervals and mock swabs containing a mixture of various female body fluids and seminal fluid were created. Portions of the post-coital swabs and the body fluid mixtures were digested in sterile tubes using the Erase Sperm Isolation Kit method. After digestion, the sample was centrifuged to pellet intact sperm cells. The supernatant was transferred from the original tube into a new tube. DNA was extracted from the supernatant containing the non-sperm fraction. Next, the nuclease was added to the original tube containing the sperm fraction in order to degrade the DNA free in solution. After the appropriate incubation period, the nuclease was inactivated and the sperm fraction was then lysed and purified. DNA was purified from both the non-sperm and sperm fractions using two methods: the Qiagen DNA Investigator Kit and organic extraction. Samples which contained mixtures of saliva, blood, and seminal fluid were subjected to the same procedure. Prior to lysing the sperm fraction, the pellet obtained from the sperm fraction was examined for the presence of spermatozoa using Kernechtrot Picroindigocarmine staining method and microscopy.

DNA was quantified using the Quantifiler® Human DNA Quantification Kit and amplified using commercially available PCR Amplification kits. The amplified products were injected on the AB 3130xl Genetic Analyzer, followed by analysis with SoftGenetics GeneMarker® HID software.

The results of the study showed that complete, single source male and female autosomal STR DNA profiles could be generated from swabs containing mixtures of seminal and female body fluids using the Erase Sperm Isolation kit. The method is cost effective and eliminates labor intensive and lengthy procedures used in standard differential extraction.

DNA, Differential, Extraction

A39 Automating the Differential Digestion Process in the Analysis of Sexual Assault Evidence Using Selective Degradation

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The goal of this presentation is to describe the methods of automating the differential digestion process using selective degradation of non-sperm DNA. The effects of using a degradative agent on sexual assault evidence samples will be discussed.

This presentation will impact the forensic science community by demonstrating that the selective degradation differential digestion process can produce high yield and high-quality DNA profiles given the optimal parameters. Successful application of this process allows the ability to process more sexual assault evidence in a shorter amount of time, aiding in decreasing the backlog in many forensic science laboratories.

Forensic science laboratories are faced with an increase in demand for sexual assault evidence analyses. Lacking sufficient analysts to perform the time-consuming, labor-intensive work required for DNA analysis, the large number of requests quickly results in an overwhelming backlog. This high demand has led laboratories to transition to automation for processing cases in a more efficient manner. While many areas of DNA analysis have adopted automation, the differential digestion process remains a time-consuming, manual task.

An automated differential digestion protocol was developed using selective degradation. The current differential digestion process requires multiple wash and centrifugation steps to remove residual epithelial DNA from the sperm fraction. The selective degradation technique replaces these labor-intensive steps by using a degradative agent, DNase I, to digest the remaining epithelial DNA. The use of DNase on evidence samples and its effect on DNA yield and DNA typing quality was assessed. Studies were performed on semen stains stored for an extended period of time (up to 60 years) and on semen samples subjected to heat, humidity, and multiple freeze/thaw cycles to evaluate the effects of DNase on environmentally compromised sperm samples. Sensitivity, reproducibility, and contamination studies were performed on a robotic liquid handler used to automate the differential digestion process. The automated protocol utilized 96-well plates for high efficiency and incorporated microscope slide preparations for the confirmation of the presence of sperm.

Initial trials of the selective degradation process resulted in lower DNA yield. Samples digested with the selective degradation process recovered approximately 30% of the sperm fraction male DNA when compared to the same sample digested with the conventional method. An explanation for the loss of sperm DNA was incomplete deactivation of DNase prior to sperm lysis. DNase activity is directly related to the divalent ion concentration so multiple experiments were performed to optimize the Mg^{2+} and Ca^{2+} concentrations. Results showed that decreasing the Ca^{2+} concentration from 125mM to 5mM significantly increased the DNA yield compared to the conventional method.

STR DNA typing for samples subjected to the initial, non-optimized selective degradation process resulted in poor DNA typing quality. DNA sample input of 1.5ng produced profiles with low peak height levels ranging from ~100-500 RFUs and peak height imbalances. The poor typing data may have been caused by inhibition and degradation. Inhibition was eliminated as a possible cause through controlled experiments. Degradation caused by DNase was tested by decreasing the amount of DNase used from 360 units to 18 units. Decreasing the amount of DNase resulted in significantly improved STR DNA typing

data. The peak height levels were approximately three times that of the same sample digested with 360 units of DNase.

Environmentally compromised sperm samples were digested using the conventional method and the selective degradation method. DNA quantitation showed no statistical difference in the DNA yield for the samples prepared using both methods. No difference in the STR DNA typing data between both sets of samples was observed. Most samples resulted in full DNA profiles with satisfactory peak heights. A few samples yielded partial profiles with low peak heights. These results were observed using both conventional and selective degradation methods, indicating that compromised sperm samples were able to withstand DNase treatment.

Sensitivity, reproducibility, and contamination studies were performed on an automated robotic liquid handler. Sensitivity studies showed that full DNA profiles were obtained from samples with DNA yield as low as 150pg (~2 sperm/3µL). In samples where no sperm were observed microscopically, the STR DNA typing data resulted in either no or few alleles detected. Quantitation data from the reproducibility studies demonstrated that the robot was very consistent in the manipulation and preparation of the samples. Contamination studies showed no signs of contamination in the automated selective degradation differential digestion process.

Through the use of the selective degradation method, automation of the differential digestion process was achieved without having to compromise on the quantity and quality of the DNA obtained.

Selective, Degradation, Automation

A40 Separation of Complex DNA Mixtures From Touch Evidence

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After attending this presentation, attendees will gain an understanding of next generation sequencing and its potential application to the forensic field through deconvolution of complex DNA mixtures from evidentiary items.

This presentation will impact the forensic science community by introducing a future capability that has the potential to separate complex DNA mixtures of five to seven individuals, including low copy contributors.

DNA sequencing technology is advancing rapidly in the form of Next Generation Sequencing, and the amount of catalogued genetic human data being generated has created an explosion of possible applications. The Bode Technology Group is currently applying the use of Next Generation Sequencing to the deconvolution of complex forensic mixtures from low copy number, degraded, and touch objects. To this end, Bode has designed, developed, and tested a field functioning forensic process for separating complex deoxyribonucleic acid (DNA) mixtures of five to seven individual sources of DNA and producing distinct profiles for each source using pyrosequencing-based deep amplicon sequencing chemistry. By combining the power of a pyrosequencingbased Next Generation Sequencing platform such as the bench topsized Roche 454 GS Jr. sequencing system with a novel forensic bioinformatic software pipeline, mixtures of seven or more individuals from mock evidentiary touch samples have been successfully sequenced and separated using multiple panels of highly multiplexed forensically relevant loci.

Data will be presented from an ongoing research effort to deconvolute mixtures of both mitochondrial DNA and nuclear Y chromosome Short Tandem Repeat (Y-STR) amplicons using a bioinformatic software suite developed through a collaboration with The Johns Hopkins University Applied Physics Laboratory. Data produced by the mixture separation software suite show highly sensitive detection of low copy contributors. When applied to mixtures of between two and seven individuals sequenced at mitochondrial hypervariable and Y-STR loci, minor contributors were successfully detected below a 1:100 ratio level, far outreaching the limit for common forensic capillary electrophoretic instruments and currently available mixture deconvolution software applications. Given a maximum number of sequences per read of between 50,000 and 120,000, there is a tradeoff among several factors such as the number of multiplexed loci, the number of contributors to a mixture, and necessary sensitivity. Thus, to develop a working understanding of the limits of this system, a series of mixture samples were created changing these variables.

Several sample types were examined including: dilution series of nascent human DNA and mock touch samples created from fingerprints (handled objects for varying degrees of time), multiple biological fluids, and substrates in complex mixtures consisting of between 1-8 different contributors at different relative concentrations. Multiplexes of the mitochondrial hypervariable regions HVI/HVII and standard Y-STR loci were created, balanced, and optimized into panels compatible with the 454 GS Jr. sequencing system. The raw data files are directly imported into the software, where all of the sequence reads at each locus are detected and then separated. Each unique sequence is binned and analyzed for error. Using a ratio-driven algorithm, alleles are re-associated to the most likely contributor of origin with associated likelihood ratio values. The system also offers the potential to detect all alleles in a mixture, determine inclusion and exclusion statistics, and even search against databases and generate match statistics accordingly. Ultimately, this system will be available for use in the course of routine forensic casework and thus can be an important future tool in the broader forensic science community.

Next-Gen Sequencing, Mixture Separation, Mixture Analysis

A41 Error Tradeoffs in Human Identity Comparisons: Determining a Complexity Threshold and Exclusion Criteria for DNA Mixture Interpretation

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This goal of this presentation is to introduce an alternative framework for making determinations of exclusion versus inclusion for DNA mixtures that is based on running Monte Carlo simulations on a probabilistic computer model of individual and mixed human genotpyes. This method is then applied to laboratory mixture data, and the results are compared with those for the simulated mixtures.

This presentation will impact the forensic science community by emphasizing how error rates associated with DNA profile interpretation are crucial to responsible determinations of a reference's exclusion or inclusion in an evidence sample. The error characterizations demonstrated in this study are useful in a number of ways. The simulated results can be used by a laboratory to inform the establishment of a preferred interpretation range by selectively optimizing between false positives and false negatives. Alternatively, after empirically determining a level of drop-out associated with evidence samples of varying starting DNA template, an informed decision regarding exclusion of a known as a potential contributor to an item of evidence will be presented. Traditionally, such samples were reported as uninterpretable or inappropriate for comparison.

DNA analysts considering a forensic evidence sample and a reference sample (e.g., from a suspect) have three options when rendering a decision with regard to the consistency between the samples: exclusion, inclusion, and inconclusive. Complicating this determination is the reality that DNA profiles originating from forensic

mixture evidence may not be fully observed due to allelic drop-out and/or the presence of overlapping alleles. Different analyst inclinations and laboratory standards exist for informing an analyst's decision; typically—and particularly for samples demonstrating some degree of allelic drop-out—less than exactly 100% allelic consistency between known and questioned samples are not automatically precluded from inclusion in an evidence sample. In tolerating some measure of absence of a reference sample's alleles in an evidence sample, the potential for two kinds of errors exists: In a case in which an individual could not have contributed to an evidence sample, there is the potential for false inclusion; in a case in which an individual could have contributed, there is the potential for false exclusion. In selecting a particular decision criterion to inform determinations of inclusion or exclusion, a tradeoff between these errors exists. A lax decision criterion minimizes false exclusions at the expense of false inclusions while a strict criterion eschews false inclusions at the expense of greater numbers of false exclusions. The relevance of a decision criterion is greatest for low-template samples and for samples that are mixtures of multiple contributors since both are likely to experience allelic drop-out and thus to occupy a potential gray area between certain exclusion and likely inclusion.

In this study, for a given level of allelic drop-out, 10,000 simulated mixtures are compared with databases of 10,000 simulated excluded and included reference individuals. In order to generate credible genetic profiles, the phenomena of allelic drop-out and profile mixing of two contributors are modeled. Comparisons between the reference and simulated mixtures at drop-out levels ranging from 0 to 0.9 are performed. Given this framework, the universe of possible decision criteria is explored. Receiver Operating Characteristic (ROC) curves, a type of analysis originally applied to assessing World War II radar performance, are adopted as a paradigm for summarizing the tradeoff of both types of errors and confirm that higher rates of drop-out result in increasingly higher incidences of error. Specifically, ROC analysis of the two-person mixtures showed that drop-out rates >0.3 result in false positive rates >0.01 and false negative rates >0.15. Here the false positive rate represents the proportion of reference standards that were incorrectly included as a potential contributor. The false negative rate is the proportion of standards that were incorrectly excluded.

ROC analysis can be used to inform the establishment of a preferred operating point by selectively optimizing between false positives and false negatives to accord with prudence. Alternatively, after empirically determining a level of drop-out associated with a particular laboratory or with evidence samples of varying starting DNA template, an informed decision can be made regarding the number of allelic discrepancies that may be tolerated before that rate of false inclusions becomes too large.

The specification of error bounds can also designate an operating region, outside of which the interpretation of an evidence profile cannot be made with the required accuracy. Whether a given evidence profile is a candidate for interpretation is a function of its associated level of drop-out. Evidence profiles shown to lie outside of the acceptable error bounds due to their level of allelic drop-out are said to fail to meet a "complexity threshold" for determinations of inclusion or exclusion. No statistics should be calculated for such samples, and the only responsible determination with respect to reference inclusion/exclusion is "inconclusive" or "uninterpretable." For evidence profiles possessing levels of drop-out that are deemed interpretable, this same "complexity threshold" can be employed to establish a laboratory's decision criteria with respect to tolerating allelic discrepancies. The resulting prescription for determining that a reference is included as a contributor to an evidentiary stain would conform with premeditated, laboratory-selected error rates and the decision regarding whether to compare the questioned sample to a reference would be made before examining a known DNA profile.

Forensic DNA, DNA Mixtures, DNA Interpretation

A42 Developmental Validation of ArmedXpert™: Forensic Mixture Deconvolution Software for Short Tandem Repeat Data

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After attending this presentation, attendees will learn of the advances made in software algorithms for deconvolution of STR data generation from forensic casework.

This presentation will impact the forensic science community by introducing changes to mixture analyses of DNA samples.

With the major advances made in capillary electrophoresis instrumentation, more precise color separation, and improved chemistries, it is even more apparent today that mixture data from forensic casework have a significant amount of information that are not fully evaluated and summarized. ArmedXpert™, a software mixture deconvolution program, is designed to automate the tedious and numerous calculations required to thoroughly review a mixed STR DNA result. ArmedXpert™ not only aids the forensic DNA analyst in these routine, time-consuming computations, but it also provides an array of significant other functions. ArmedXpert™ was subjected to a rigorous validation study where it was evaluated with known mixed data sets of two- and even three-person mixtures. At the end of the study, actual casework data files from adjudicated cases were evaluated and compared to the submitted final case reports.

ArmedXpert™ allows the user to check ladders, to check controls, to detect possible stutter, to perform matching between evidence samples and references, and to evaluate possible contamination with staff profiles. The software is designed to perform CODIS functions, conduct mixture interpretation with two to three contributor mixtures, view simulated electropherograms, chart data, perform various biostatistical analyses for single and multiple source samples, and print and save data. The stated functionality of each of these features was confirmed during the developmental validation process.

The developmental validation study of ArmedXpert™ entailed testing of three main software specifications: compatibility, QC checks and matching, and interpretation. For compatibility, different operating systems were subjected to the software to verify the ability of the software to perform as expected. All menus and functions were subjected to verification testing. In addition, different data files were imported to verify successful upload and functionality. The control checks, formatting of output files, and comparisons demonstrated full utility. Mixture interpretation tools were evaluated for display features, accurate and reproducible calculations, and application of defined thresholds. In addition, all statistical calculations were confirmed by manual testing and comparison to other software programs. The output reporting and CODIS CMF files were also verified.

Lastly, the software was evaluated for applying the appropriate thresholds and stutter and color-coding features as described. Significant studies were conducted to confirm the correct reporting of all allele calls. The Mixture Interpretation section of ArmedXpert™ produces a list all possible combinations for each locus based on the user-defined thresholds. This list provides the proportion of DNA of the minor and major contributors of the mixed sample. The graphical visualization tool provides the user with an easy visual assessment of the proportions identified per locus for the mixed result. The combinations, thresholds, proportions, and bar chart tool were all tested with known mixtures at varying concentrations.

ArmedXpert™ is a software program that can be easily adapted and implemented into the forensic analyst's toolbox. This program has many features that will assist forensic analysts to fully evaluate and summarize their data. ArmedXpert™ can be used as a stand-alone program or can support DNA analysts in the arduous task of mixture interpretation, and seamlessly import data for statistical evaluations. This developmental validation study demonstrates that the software meets the defined specifications and performs as expected.

ArmedXpert, Deconvolution, Mixture

A43 Large-Scale Evaluation of ArmedXpert™ for 2 - 4 Person DNA Mixture Analysis

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After attending this presentation, attendees will understand the effectiveness of ArmedXpert's™ ability to deconvolute Short Tandem Repeat (STR) DNA mixture data.

This presentation will impact the forensic science community by demonstrating the accuracy, effectiveness, and limits of the ArmedXpert™ program.

ArmedXpert™ is an Excel-based software program created with the purpose of assisting DNA analysts in deconvoluting two-, three- and four-person DNA mixtures. This software program uses Short Tandem Repeats (STR) and Y-STR DNA profiles, and displays the possible combinations for the various contributor scenarios. The effectiveness of this program relies on statistical probabilities and subjective interpretation from the user. The main goal of this research is to determine how accurate and effective ArmedXpert™ is at assisting forensic scientists to deconvolute DNA mixtures under varying concentrations and conditions.

In this study, two-, three-, and four-person DNA mixtures were generated and initially profiled by the Boston University School of Medicine. This pilot study evaluates the examiners' ability to interpret a DNA mixture profile from a mixture data set. The Boston University study contains over 2,000 mixture combinations, which were generated with various contributor numbers, concentrations, injection volumes, and amplification kits. The effectiveness of ArmedXpert™ was determined by analyzing the examiners' ability to use the software to correctly identify allele calls, determine the major, minor, and number of contributors, and the statistical random match probability (RMP) of each contributor when compared to the known Boston University data set. The examiners' input was entered into an spreadsheet-based macro program that calculates the partial genotype accuracy, residual frequency of the known and calculated RMPs, and the failure rate of missed allele calls. This spreadsheet macro will also determine if the calculated allele calls from the DNA analyst match the alleles from the Boston University data set, and if an incorrect allele was called. The partial genotype accuracy calculates the DNA analysts' ability to call the correct alleles when compared to the known DNA mixture profile. An average of these metrics will be calculated to determine the extent that ArmedXpert™ assists DNA analysts at mixture interpretation when compared to analysts that deconvolute without software assistance. Mixture interpretations with incorrect allele calls will be averaged separately from the correct allele calls. Statistical analysis, such as a chi-squared test, will be performed on the overall results to determine how effective ArmedXpert™ is at assisting DNA analysts at deconvoluting DNA mixtures.

In the future, a large-scale study of DNA examiners will be conducted to determine the overall metrics of the mixture evaluations. These metrics will be used to determine if ArmedXpert™ can effectively assist DNA analysts in interpreting DNA mixture analysis. Because there is no universal standard for analysis of DNA mixture, the results from the study may vary depending on the experience of the DNA analyst. The effectiveness of ArmedXpert™ may be influenced by the DNA analysts' familiarity with the software program and their ability to determine the major, minor, and number of contributors.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or reflecting the views of the Department of the Army, the Department of the Navy, or the Department of Defense.

ArmedXpert™, DNA Mixtures, Error Rates

A44 Quantitative PCR for Rapid Gel Electrophoresis-Based Pre-STR Mixture Detection

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After attending this presentation, attendees will learn a potential method for a screening technique to identify mixtures at the qPCR stage of DNA analysis.

This presentation will impact the forensic science community by introducing a quick screening method to identify a mixture DNA profile prior to STR amplification.

Forensic DNA units currently do not have a way to accurately determine if a mixture is present prior to the CE analysis stage. This can be detrimental to labs, like the ATFE, that only have a limited sample amount with which to work. When DNA samples are below 2ng at ATFE, half of the sample is used for amplification. If the Identifiler™ STR analysis shows a mixture profile with all the allele peaks below threshold, then the lab does not have enough remaining sample to re-amplify using increased DNA input. If the lab had a procedure to suggest a sample is a mixture early in the process, then ATFE would be able to make key adjustments to their amplification technique in advance. For example, the amount of DNA sample amplified could be increased in order to raise the minor contributor alleles above thresholds. Additionally, examiners could further concentrate samples or increase injection times on the genetic analyzer to allow for improved results. This study is aimed to develop a modified quantitation assay that would allow for mixture detection pre-STR amplification. If successful, identifying a mixture at the quantitation stage would allow for adjustments to be made with a sample prior to amplification and analysis, such as using the full sample elution instead of the standard half sample or combining swabs taken from the same surface.

In this study, the goal was to develop a multiplex qPCR assay that would incorporate either two Single Nucleotide Polymorphism (SNP) markers or D1S80 into the existing Investigator® Quantiplex quantitation reaction for mixture identification. SNPs were chosen that have a minor allele frequency above 10% to increase the likelihood of detecting the minor component of a mixture. These SNPs included rs5030240, rs385780, rs433342, and rs4540055. Primers were designed using Primer BLAST through NCBI. These primers were designed such that the three different alleles would be able to be distinguished from one another when run on a gel. This was achieved by adding poly-T tails of varying lengths to the end of the primer containing each of the different alleles. D1S80 was also chosen based on its well-established PCR chemistry and primer sets. The 16 bp repeat with over 22 alleles together with a high level of heterozygosity across populations increased the likelihood that a mixture DNA profile would be seen. The selected primers were tested for visibility and discrimination with ten DNA samples using the Lonza FlashGel® system. The Lonza FlashGel® system using precast 2.2% agarose DNA cassettes can run at high voltages, and complete DNA migration within 2 - 7 minutes.

Product gels showed bands at the expected sizes for all four SNP markers, and all reagent blanks were clear. However, resolution of the alleles was not sufficient for allelic discrimination. The D1S80 gels showed bands at expected sizes and alleles could be discriminated from one another. Mixture samples amplified with D1S80 could also be discriminated on the gel system; however, the D1S80 primers appeared to interact unfavorably with the Investigator® Quantiplex reaction when incorporated into the qPCR reaction. Further studies should be conducted to look for alternate ways of incorporating these or other markers into the quantitation reaction. Additionally, primer redesign or new detection methods are possible development techniques to improve current allele discrimination. Ultimately, this identification would result in a decrease in sample consumption issues as well as a time savings for forensic DNA labs when analyzing casework contact DNA.

qPCR, DNA Mixtures, SNP Markers

A45 Comparison of Two Immunochromatographic Test Strips for the Detection of PSA

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After attending this presentation, attendees will have gained further insight into the sensitivity and specificity of two commercially available immunochromatographic tests for the detection of Prostate Specific Antigen (PSA also known as p30). Of particular interest are potential concerns regarding the specificity and sensitivity of immunological-based assays in forensic-type casework. The detection of endogenous p30 from controlled vaginal swabs raised potential concern for the application of these methods in the forensic community. Furthermore, some assays demonstrated the loss of cellular material prior to microscopic analysis and/or downstream analysis.

This presentation will impact the forensic science community by raising awareness of the limitations that exist with current immunochromatographic test strips employed by forensic practitioners.

The brentamine blue spot test for acid phosphatase (AP) is often used as a preliminary screening test for suspected semen stains. Given its limited specificity, suspected semen stains must be analyzed microscopically for the presence of spermatozoa. If spermatozoa are not present, suspected semen samples may be tested for the presence of PSA in conjunction with microscopic examination. PSA is present in high concentrations in seminal fluid but is also found in lower concentrations in amniotic fluid, breast milk, vaginal secretions, and female urine. Current testing methods that exist for the detection of PSA, such as double immunodiffusion, are both time consuming and lack sensitivity. Internal validation of several commercially available immunochromatographic tests for the detection of PSA was conducted. Validation of two independent assays was performed to evaluate the sensitivity, reproducibility, stability, and specificity of each test.

Known semen samples were detected down to a 10^{-4} dilution in both test strips, with reproducibility of positive results from a 10^{-3} dilution. Positive results for the AP test were observed in dilutions down to 10^{-2}. Confirmatory identification of spermatozoa was detected in samples containing dilutions down to 10^{-4} through microscopic analysis. The use of deionized water as an extraction buffer had no adverse effects on test sensitivity or specificity results. Specificity of the tests was demonstrated through negative results in all body fluids tested with the exception of male urine and vaginal secretions. The addition of more vaginal swabs from females with complete sexual and menstrual cycle history was obtained. These samples demonstrated a surprising false positive rate of approximately 30% for PSA for both tests. Chemically treated samples were used to demonstrate the stability of both test strips. One of the immunochromatographic strips indicated significant loss of cellular material, both epithelial cells and spermatozoa, when the test extract was used for microscopic detection of spermatozoa in downstream testing.

Based on these analytical results, both of the immunochromatographic assays under evaluation demonstrated comparable sensitivity and specificity for the detection of PSA from forensic-type samples. The loss of cellular material that was observed microscopically in this study can have serious consequences in DNA investigations and can significantly impact a forensic case. The lack of specificity for PSA as a seminal marker raises concerns regarding the implementation of immunochromatographic-based tests for the detection of PSA in forensic-type casework.

Seminal Fluid, Vaginal Secretions, Rapid Test Strips

A46 DNA Quantification by Real-Time PCR (qPCR) and Short-Tandem Repeats (STRs) Amplification Results

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After attending this presentation, attendees will understand: (1) principles of genetic analyses on forensic samples; (2) the importance of a valid quantification technique; and, (3) the issues related to the analysis of low template DNA samples.

This presentation will impact the forensic science community by highlighting the importance of the DNA quantification step in forensic casework in spite of the increasing sensitivity of last generation commercial kits for STR analysis that allow the detection of allelic peaks from extremely low DNA quantities (even with concentrations far below the limit of detection for the specific quantification kit).

Determining the amount of DNA in a forensic sample is fundamental for PCR-based analyses because if on one hand an excessive amount of template may cause the appearance of additional or out-of-scale peaks, on the other hand, a low quantity can cause the appearance of stochastic phenomena affecting the PCR reaction and the subsequent interpretation of typing results. In the common practice of forensic genetics laboratories, the quantification results provided by qPCR assume the role of "boundary line" between the possibility for a given DNA sample to be subjected or not to the subsequent analytical steps, on the basis of an optimal amount of DNA in the range indicated by the manufacturer of the specific commercial kit.

However, some studies have shown the possibility to obtain STR typing results even with an extremely low DNA concentration or, paradoxically, equal to zero. Regardless of the amount of DNA used for the quantification of the testing sample, certain software is able to use the standard curve to calculate concentration values far below the manufacturer's reported optimal detection limit (0.023ng/µL). Consequently, laboratories have to face the critical decision to interrupt the analyses, giving up the possibility to obtain a genetic profile—although partial—or to try the amplification of the extract with the awareness of the interpretation issues that this implies.

The quantification results obtained by qPCR performed on numerous samples from specimens of forensic interest, subjected to DNA extraction using magnetic beads will be presented. Following the quantification step, the extracts were subjected to DNA amplification and STR typing using last generation commercial kits. Samples that showed quantification values below the limit of detection for the method were included in the analysis in order to check the existence of a correlation between the DNA quantification results by qPCR and the possibility of obtaining a genetic profile useful for identification purposes.

In spite of the increasing sensitivity of last generation commercial kits for STR analysis, as demonstrated by the ability to detect allelic peaks from extremely low DNA quantities (with concentrations far below the limit of detection for the specific quantification kit, even corresponding to 0 or "Undetermined"), the results obtained show a correlation between qPCR quantification values and STR typing results. Thus, the qPCR method is confirmed as being a useful and valid instrument for both qualitative and quantitative evaluation of genetic samples for human identification purposes.

Quantification, Real-Time PCR (qPCR), Short Tandem Repeats

A47 Evaluation of Half Reaction Volumes of the AmpFℓSTR® Identifiler® Plus Forensic Amplification Kit in STR Analysis

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After attending this presentation, attendees will have a better understanding of how reduction of reaction-mixture volumes of the multiplex PCR system IdentifilerPlus® Forensic Amplification Kit from Applied Biosystems affects amplification of the Short Tandem Repeat (STR) markers in the kit and the corresponding interpretation of forensic DNA profiles from these markers.

This presentation will impact the forensic science community by demonstrating the effectiveness of STR amplification with the IdentifilerPlus® kit when the reaction volume is reduced by half. Utilization of half-reaction volumes of the IdentifilerPlus® kit will provide a significant savings to a forensic science laboratory; however, these data show that caution should be exercised when interpreting genetic profiles generated from low-DNA input quantities.

Many forensic science laboratories have transitioned to using the AmpFℓSTR® IdentifilerPlus® Forensic Amplification Kit for STR analysis to streamline evidence processing by increasing sensitivity, increasing resistance to PCR inhibitors, and reducing the time for DNA amplification. Each IdentifilerPlus® kit contains the reagents to amplify 16 STR loci, including the 13 core CODIS markers, for approximately 200 samples. At a cost of over $17.00 per sample, using full reaction volumes for every sample may be unnecessarily expensive. This study was performed to evaluate the effectiveness of DNA amplification when the total volume of IdentifilerPlus reactions is reduced by half.

Purified DNA samples containing heterozygous alleles at all loci with the exception of amelogenin (female) were amplified in duplicate on an ABI 9700 thermocycler using the recommended 28-cycle protocol. Samples were amplified with the IdentifilerPlus kit using half the volume of reagents and template DNA as the volume recommended by ABI, for a total reaction volume of 12.5µl. They were then injected on an Applied Biosystems™ 3130xl Genetic Analyzer for 2, 4, or 6 seconds, and the resulting data were analyzed with GeneMapper ID. A DNA target range of 0.001-1.25ng was assessed to determine the effects of half reaction volumes on the sensitivity of amplification, locus detection, stochastic threshold, and peak height ratios (PHR). The results of these studies were also compared to the values obtained from the previous internal validation of the IdentifilerPlus system at the full reaction volume of 25µl.

For the amplification sensitivity study, the high DNA input samples in half reactions produced similar peak height values when compared to full reaction volumes. The target DNA input for half reaction volumes of the IdentifilerPlus kit was determined to be 0.5 – 1.0 ng, the same quantities recommended and validated for full reaction volumes. Importantly, the stochastic threshold increased from 145 RFU for full reactions to 325 RFU for half reaction volumes, making half reactions much less useful for evidence samples.

The PHR for half reactions were similar to those seen in our previous study of the IdentifilerPlus® kit using full reaction volumes. These data suggest that PHR is not affected by using half reaction volumes with greater DNA input amounts, i.e., more than 0.2ng. However, with lesser DNA target amounts, i.e., less than 0.2ng, the percentage of heterozygous peaks with PHR >60% was consistently greater than 80%, while at half reaction volumes, the percentage of heterozygous peaks >60% PHR was lower, in the 60-80% range.

The increase in stochastic threshold and PHR variability at lower DNA quantities suggest that input amounts of 0.75ng or higher are more useful and more reliable for half reactions. Results of tests for reproducibility, accuracy, and precision will be presented. The Harris County Institute of Forensic Sciences processed 1,560 known DNA samples from January through May 2012. The use of half reaction volumes for IdentifilerPlus would provide a savings of over $13,000 within this time period alone.

In conclusion, the use of half reaction volumes of the IdentifilerPlus amplification kit may be a viable option for processing known forensic DNA samples where an unmixed sample and a high quantity of DNA is expected. This would result in a significant cost savings for the laboratory. For unknown samples, the doubling of the stochastic threshold makes the use of half-reaction volumes unsuitable.

Identifiler® Plus, Half Reactions, Amplification

A48 High Throughput SNP Typing Based on Invader® Assay on Integrated Fluidic Circuits

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After attending this presentation, attendees will understand the effectiveness of a high-throughput multiplex SNP (single nucleotide polymorphism) typing system utilizing integrated fluidic circuits (IFCs), which are fluidic lines designed on a detection disc forming 2,304 reaction chambers.

This presentation will impact the forensic science community by providing an approach for multiplex invader assay SNP typing in thousands of reactions. Dozens of SNP loci in dozens of DNA samples can be quickly detected at low cost.

SNP typing has advantages in forensic identification, because SNPs are abundant in genomic DNA and are easily detected using an automated high-throughput system. The primary benefit of SNP typing is more successful typing of degraded samples compared with other methods, including short tandem repeat (STR) typing, because of their small target size. SNPs, however, are bi-allelic and large numbers of loci must be analyzed to increase the power of discrimination. To obtain a comparable discriminatory power to that of the commonly used fifteen loci STR typing kit, more than 40 SNP loci must be typed. In addition, the analysis of this many loci should be simple, cheap, and rapid. Common SNP typing procedures, such as the TaqMan assay, the cycling probe assay, the single primer extension assay, or the Invader assay, cost a few dollars for each locus. Thus, typing 40 loci would cost more than a hundred dollars.

In this presentation, high throughput and low cost multiplex SNP typing using the Invader assay in IFCs on a BioMark HD System produced by the Fluidigm Corporation are introduced. In this system, reactions proceed in a few nanoliters of solution enclosed in small chambers, rather than the commonly used microliter-scale reaction tubes. The flow of reaction mixtures and DNA sample solutions are automatically controlled and filled in separated chambers by air pressure, without interdiffusion. The volume of each reaction mixture is very small and thus the total cost of analysis is low. In the device, the temperature of the reactions is controlled and the fluorescence of each chamber is detected and recorded in real time. Using this system, we attempted to analyze 32 SNPs that had been validated in our previous study.[1]

DNA samples were collected from 48 individuals who had given their informed consent to our experiments. Their SNPs were analyzed in our previous study and confirmed by direct sequencing. The Invader assay for the amplified products of the 32 SNP loci by multiplex PCR were carried using Universal General Purpose Reagent (Third Wave Japan Co.) injected into the chambers on the detection disc. The intensities of two types of fluorescence, FAM and Yakima Yellow, in each chamber were detected every 20 seconds during the reaction, which used isothermal heating at 63°C for 30 minutes. The genotypes of each locus were determined from the increasing fluorescence curve of the chart in which both fluorescence intensities were plotted, as reported previously.[2] Triplicate typing for each sample was carried out to verify the results. Using the IFCs system, high-throughput and low cost multiplex SNP typing could be achieved. The reaction and detection time for 48 samples in the device was only approximately 40 minutes, not including one hour of PCR amplification and the 30 minutes taken to

inject the samples into the chip. The cost for each SNP locus was less than 25 cents. Furthermore, this system can also perform digital PCR as another application of IFCs. Digital PCR is a quantitative analysis method commonly used in gene expression studies. It is expected that the application of IFCs will be effective for resolving the problem of DNA mixtures in forensic casework analyses, when used in the quantitative detection of different alleles.

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SNP, Invader Assay, Fluidic Circuits

A49 Validation of the Applied Biosystems® 3500 Genetic Analyzer With a Comparison of the Identifiler® Plus and PowerPlex® 16 HS Amplification Kits

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The goal of this presentation is to demonstrate the ability of the Applied Biosystems 3500 genetic analyzer to produce complete and accurate forensic DNA profiles with a variety of sample types under a variety of different conditions. It will also provide a comparison of two commonly used amplification kits from the two major manufacturers of forensic analytical supplies for consideration by forensic laboratories. A denaturation study shows the ability for laboratories to validate procedures without the denaturation and snap cooling step prior to a run on an instrument such as the AB 3500. This has the potential to increase efficiency in forensic casework analysis. This validation can also serve as a starting point for future validations of the AB 3500 genetic analyzers.

This presentation will impact the forensic science community by validating the Applied Biosystems 3500 genetic analyzer and the comparison of the PowerPlex® 16 HS and Identifiler® Plus amplification kits on this instrument will provide the forensic science community with information that will allow laboratories to make informed decisions about the kits and equipment that would be best suited to their needs.

Applied Biosystems® 3500 series of genetic analyzers is the newest technology available for forensic casework analysis and has not yet been widely adopted by the forensic community. As such, there are limited studies available that involve the performance of amplification kits commonly used on these instruments. The Anne Arundel County Crime Lab is currently upgrading from an AB 310 genetic analyzer to an AB 3500 genetic analyzer. The internal validation of this AB 3500 included a comparison of the PowerPlex® 16 HS and Identifiler® Plus amplification kits using casework samples and the manufacturer's recommended protocols to determine if one had any advantages over the other, when used in conjunction with the AB 3500. Analytical and stochastic thresholds were calculated for both a 7-second and a 15-second injection time, which were validated for casework analysis on this AB 3500. Precision, contamination, sensitivity, concordance, reproducibility, and stutter studies were also performed during this validation. The AB 3500 generated complete and accurate forensic DNA profiles with both kits and both injection times over a wide range of DNA target amounts at amplification. However, the AB 3500 was able to generate more complete profiles for lower level single source samples and mixture samples using PowerPlex® 16 HS, when the same target amount of DNA was used at amplification in each kit. Powerplex® 16 HS was also shown to generate higher peaks across each dye channel on the AB 3500 when compared to Identifiler® Plus. Aside from this difference in sensitivity, there were no significant differences in performance between the PowerPlex® 16 HS and Identifiler® Plus amplification kits on the AB 3500. A denaturation and snap cooling study showed that the denaturation and snap cooling step in the laboratory's standard operating procedures had no effect on the data produced by the AB 3500 with either kit. As a result of this validation, the Anne Arundel County Crime Lab will continue to use the PowerPlex® 16 HS amplification kit for casework analysis on their newly validated AB 3500 with a standard operating procedure that does not include the denaturation and snap cooling step prior to an instrument run. Extended use of the POP 4 polymer pouch was also investigated beyond the recommended 7 days on the instrument after opening. Storing the POP 4 polymer in the refrigerator after opening, when it is not in use on the instrument, was shown to extend the useful life of the polymer by several weeks. Future studies may include manipulation of the amplification cycles with Identifiler® Plus to see how the sensitivity of this kit can be improved for lower level single source samples and mixture samples. There is also a tendency for the D13 and D5 loci to dropout more frequently in lower level samples than other loci with both PowerPlex® 16 HS and Identifiler® Plus on both the AB 3500 and the AB 310, which was used in this validation for concordance studies. Future studies could be undertaken to determine the cause of this imbalance.

AB 3500, PowerPlex® 16 HS, Identifiler® Plus

A50 Internal Validation of the AmpFℓSTR® Yfiler™ Amplification Kit on a Life Technologies™ 3130 Genetic Analyzer

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After attending this presentation, attendees will understand the process of an internal validation of the AmpFℓSTR® Yfiler™ PCR Amplification Kit on a Life Technologies™ 3130 Genetic Analyzer and the efficiency of the amplification kit to reliably amplify and produce complete and accurate Y-Short Tandem Repeat (Y-STR) profiles. The following properties are taken into account: threshold, sensitivity, contamination, precision, concordance, reproducibility, male-female mixtures, male-male mixtures, and stutter.

This presentation will impact the forensic science community by increasing throughput of casework and reducing backlog, which is one of the main problems many laboratories face. By utilizing the Y-STR selectivity to amplify STRs, the AmpFℓSTR® Yfiler™ PCR Amplification Kit can be utilized in mixture samples to help reduce interpretation time. Before implementing the AmpFℓSTR® Yfiler™ PCR Amplification Kit, an internal validation is necessary to ensure that performance in the laboratory is concordant with the performance demonstrated by the manufacturer.

The successful validation of the AmpFℓSTR® Yfiler™ PCR Amplification Kit may reduce the time spent on mixture interpretation and can increase the throughput of the Prince George's County Police Department DNA Laboratory. Y chromosomal short-tandem repeat (Y-STR) amplification is of interest at Prince George's County Police Department DNA Laboratory due to the overwhelming number of cases with complex mixtures that the analysts encounter annually. Y-STR amplification targets the male component, the Y chromosome, and can be utilized in cases where the male component is of interest. The AmpFℓSTR® Yfiler™ PCR Amplification Kit is a multiplex assay that amplifies 17 loci located on the Y chromosome: DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385a/b, DYS393, DYS391, DYS439, DYS635, DYS392, Y GATA H4, DYS437, DYS438, and DYS448. The resulting profile is a human male haplotype. In autosomal amplification, loci from several different chromosomes are amplified in

one reaction, and in the case of mixture samples can be difficult to interpret. Using a male specific kit, such as the YFiler™ kit, male profiles can be isolated and aid in interpretation, especially in conjunction with a genotype produced from autosomal analysis. The internal validation of the Yfiler™ kit followed the SWGDAM validation guidelines along with a comparison of analytical threshold calculations. The analytical threshold calculations were completed using two different sample type runs using a 5second capillary electrophoresis injection. The samples included capillary electrophoresis run negative samples and YFiler™ amplified samples (including samples, positive controls, and reagent blanks). For the run negative analytical threshold calculations, the average and standard deviation of the baseline height (disregarding internal size standard pull up or known artifacts) and separated by dye channel. The analytical threshold using YFiler™ amplified samples was calculated by evaluating average height and standard deviation of the baseline between allelic peaks (disregarding artifacts such as pull up and stutter). Through both sample type calculations, the optimal analytical threshold was found to be 150 relative fluorescent units (RFU) for a 5-second injection time. A 10-second injection time also utilized a 150 RFU analytical threshold, while a 200 RFU analytical threshold was utilized for a 15second injection time. Full profiles were produced for a 5-second injection time from a DNA input concentration of 2.0ng/µL to 0.250ng/µL, with full profiles produced from 0.125ng/µL in six of 10 samples, and an average of 11 loci for 0.0625ng/µL. For a 10 second injection time, a DNA input concentration from 1.0ng/µL to 0.250ng/µL produced full profiles, with full profiles produced from 0.125ng/µL in all but one of 10 samples, and an average of 14 loci for 0.0625ng/µL. Full profiles were produced for a 15-second injection time from 0.50ng/µL to 0.125ng/µL, with full profiles produced from 0.0625ng/µL in two of ten samples. In the male-male mixtures, a full profile from the minor male component was identified up to a 1:5 ratio and a 1:1:1 male mixture produced full profiles for all male contributors. In the female-male mixtures, a full Y-STR profile was obtained at ratios up to 1:1. Inhibition was observed at ratios above 1:1, with an average of six loci called in the 1:100 male to female ratio. A full male profile was produced from a 1:1:1 male to female to female mixture. The studies illustrate that the Yfiler™ kit successfully amplifies evidence samples from adjudicated cases, is male specific, and precise.

Y-STRs, Internal Validation, Y-Chromosome

A51 Validation of the Applied Biosystems® 3500xL With PowerPlex® 16 HS

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After attending this presentation, attendees will understand the procedures of the internal validation performed at the Philadelphia Police Forensic Science Bureau on the Applied Biosystems® 3500xL with PowerPlex® 16 HS. Attendees will learn which settings were selected to optimize the use of this chemistry with the instrument as well as the results of the various studies performed.

This presentation will impact the forensic science community by the sharing of information in regards to the validation of a relatively new platform to the field of forensics, the Applied Biosystems® 3500xL Genetic Analyzer. This instrument will soon replace the Applied Biosystems® 3130 Genetic Analyzer as the primary capillary electrophoresis instrument used in forensic DNA laboratories. This validation also incorporated a frequently used amplification chemistry, Promega Corporation's PowerPlex® 16 HS.

An important aspect of forensic science is the need to validate new chemistries and instrumentation for use with STR analysis. An internal validation must be performed on these new methods and technologies prior to implementation in laboratory standard operating procedures. This validation must demonstrate the ability of the procedure to obtain reliable results, the ideal conditions to obtain these results, and the limitations for this new procedure. An internal validation was performed for the Philadelphia Police Forensic Science Bureau through the National Institute of Justice Technical Assistance Program on the use of PowerPlex® 16 HS amplification chemistry with the Applied Biosystems® 3500xL Genetic Analyzer. The 3500 Genetic Analyzer is a relatively new platform to the field of forensic science and offers many advantages over the previously used Applied Biosystems® 3130, such as, an improved mechanical pump, new laser technology, more consistent temperature control, prepackaged consumables, and reduced power requirements. Data files are also saved in .HID format and analyzed with GeneMapper® ID-X (Applied Biosystems). The PowerPlex® 16 HS amplification chemistry, released in 2009, added hot-start Taq directly to their mastermix, eliminating the need to purchase this reagent separately, an advantage over the previously used PowerPlex® 16 kit. PowerPlex® 16 HS also offers the ability to perform direct amplification procedures, as well as an increased ability to perform in the presence of inhibitors.

This internal validation incorporated a variety of studies to demonstrate the reliability and reproducibility of this instrument with the PowerPlex® 16 HS chemistry. These studies included the determination of an appropriate analytical threshold through SWGDAM approved methods, a stochastic threshold, an injection time and voltage that provided optimal peak heights and peak height ratios, a sensitivity range to provide the optimal input amount of target DNA, heterozygosity ratios, and stutter percentages for comparison to manufacturer-recommended values. Studies were run simultaneously on both the 3130xL and 3500xL instruments for the duration of the validation study. The results from the 3500xL were compared with data collected from the 3130xL to demonstrate concordance. Results collected for various other studies were also compared between the two platforms including stutter ratios and mixture interpretations. Samples were also analyzed to demonstrate reproducibility across multiple amplifications and runs, and were compared with the expected results from previously analyzed non-probative casework samples. These non-probative casework samples demonstrated various extraction methods and substrates typically encountered in the Philadelphia Police Forensic Science Bureau. The comparison of these results demonstrates instrument concordance.

Overall, results from the 3500xL were observed to contain peaks of a greater intensity when compared to the 3130xL. The instrument also provided accurate profiles with few artifacts across a wide range of input target DNA. Based on the findings of these studies, specific settings were recommended to be incorporated into the standard operating procedure of the Philadelphia Police Forensic Science Bureau. These settings included a set analytical threshold across all dye channels, a stochastic threshold value to assist in the determination of true homozygote peaks, an optimal target DNA range, laboratory specific stutter ratios, and mixture interpretation guidelines. The instrument produced reliable results with the PowerPlex® 16 HS amplification chemistry and the use of this chemistry with the 3500xL was recommended for future use.

PowerPlex® 16 HS, 3500xL, Validation

A52 Validation and Comparison of the AmpFℓSTR® Identifiler® Plus PCR Amplification Kit to Identifiler®, MiniFiler™, and Yfiler® for the Pinellas County, Florida Forensic Laboratory

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After attending this presentation, attendees will gain an understanding of the Identifiler® Plus amplification chemistry's performance in various studies as compared to the Identifiler®, MiniFiler™, and Yfiler® chemistries.

This presentation will impact the forensic science community by providing information about capabilities and limitations of Identifiler® Plus under various conditions and on different instruments. By comparing this amplification chemistry to other available chemistries, forensic laboratories will have advanced insight into which chemistry may be best suited for their specific needs.

Using these protocols, amplification of DNA samples must occur prior to capillary electrophoresis in order to generate the desired human identification profile required for presentation in court proceedings. The type of amplification chemistry used in this process can affect the profile obtained from the sample. The Identifiler® Plus amplification chemistry from Applied Biosystems was selected for this study as it contains a reportedly improved master mix, with AmpliTaq Gold® DNA Polymerase already added to the mix as well as an enhanced formula to reduce Polymerase Chain Reaction (PCR) inhibition of samples. The kit is designed to provide improved sensitivity to enable use with low template DNA samples, while also allowing for more effective analysis of mixture samples. Identifiler® Plus uses fluorescent multi-color dyes to analyze 15 loci with alleles with overlapping size ranges. It follows the same design as Identifiler®, but is an improvement on the MiniFiler™ chemistry, which only amplifies nine loci. Yfiler amplifies 17 Y-STR loci, so direct comparisons could only be made for certain studies.

An internal validation of Identifiler® Plus was performed for the Pinellas County Forensic Laboratory in Largo, Florida. Eight validation studies were performed, including accuracy, precision, recovery, linearity/sensitivity, range, mixture, carryover, and ruggedness. Accuracy was determined from non-probative samples that simulated casework, mixture samples created from positive controls, and all positive controls used in the validation. Precision was measured from the standard deviations of 48 allelic ladders and the 250 base pair (bp) peak of all samples in the validation. A sensitivity study was used to measure linearity, in that a range of DNA concentrations were amplified in order to establish the optimal concentration for successful amplification. 9947A and 007 control samples were used to create mixture ratios ranging from 19:1 to 1:19. Ruggedness was determined by amplifying the samples using three different thermal cyclers. All negative controls used in the validation were analyzed for contamination. The results were compared to the results of samples amplified with Identifiler® as well as results from previous Identifiler®, MiniFiler™, and Yfiler® validations. All samples were amplified for 28 and 29 cycles to determine the ideal PCR cycle number for Identifiler® Plus. Additionally, the limit of detection and sensitivity samples were run on three different genetic analyzers (two 3130 genetic analyzers and one 3130xl genetic analyzer) to ascertain any discrepancies between the instruments.

The results of the validation supported the use of 28 PCR cycles with Identifiler® Plus. The samples displayed greater sensitivity than Identifiler® and were comparative to MiniFiler™ and Yfiler®. Results of the precision study fell within manufacturer recommendations. Samples demonstrated 100% accuracy and no contamination was present in any of the negative controls used in the validation. Samples run at different times and on different thermal cyclers were consistent with expected results and with each other. Mixture samples could be resolved and full profiles of the minor contributor were generated down to 6:1 and 1:9. The samples run on the 3130xl genetic analyzer displayed greater sensitivity than samples run on the 3130 genetic analyzer, but limit of detection stayed the same for both instruments.

Identifiler® Plus, Validation, Amplification

A53 Detection of Differentially Methylated Parental Allele in Imprinted Gene SNRPN Using MS-SSCA

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After attending this presentation, attendees will learn a new method to identify differentially methylated parental alleles.

This presentation will impact the forensic science community by using MS-SSCA in dried blood spots to identify differentially methylated parental alleles.

In a classic forensic paternity test, the obligatory gene often cannot be determined in motherless cases, or when the mother and child share the same heterozygous genotype. Recently, the combination of Single Nucleotide Polymorphism (SNP) typing and genomic imprinting has shown promising potential in paternity testing or personal identification. SNPs are the most abundant type of human polymorphism, and became valuable markers for forensic identification and paternal testing. Determining the parental origin of SNPs is helpful to some cases. Genomic imprinting is the phenomenon where the two alleles of some genes are differentially expressed according to their parental origin. DNA methylation pattern of an imprinted locus is unique to each allele. The parental origin of specific methylation status at the imprinted loci provides a new means to detect the obligatory gene without genealogical analysis. Classic techniques to detect the differentially methylated parental alleles are methylation-specific PCR (MSP) and post-digestion PCR. The established procedures; however, often require relatively large amounts of DNA. In daily practice, samples submitted for analysis might contain very small amounts of poor quality material, as is often the case with forensic stain samples, while dried blood spots have become preferred forensic samples in many forensic laboratories, as they are easy to collect, transport, and store. In this study, a new technique was developed that only requires small amount DNA to identify the obligatory gene in forensic paternity testing. First, a modified, more efficient method of bisulfite genomic sequencing in dried blood spots was tested. Briefly, the genomic DNA extracted from 3mm dried blood spots using QIAamp micro kit was treated with sodium bisulfite (EpiTect) followed by methylation-specific PCR (MSP) and Sanger sequencing. The imprinted region the gene, small nuclear ribonucleotide protein N (SNRPN) was examined for differential methylation. The data shows efficient DNA extraction and full conversion of unmethylated cytosine to uracil. Second, the methylation-sensitive single-strand conformation polymorphism (MS-SSCA) method to detect the parental origin of the allele of SNP locus rs220030 (C/T) was used. The SNP locus rs220030 is found in the promoter region of the maternal imprinted gene SNRPN. Rs220030 (C/T) in maternal imprinted gene SNRPN was typed using the TaqMan SNP Genotyping assay. The methylated status of maternal and paternal alleles were determined by MS-SSCA on four family trios with heterozygous children. The results have shown that MS-SSCA has great potential in the discrimination of differentially methylated parental alleles of imprinted genes in forensic paternity testing. This study suggested that bisulfite treatment of genomic DNA, combined with classical non-denaturing polyacrylamide electrophoresis can become a new parental origin determination method in imprinted genes for forensic purposes.

DNA Methylation, Methylated Parental, MS-SSCA

A54 Evolving Strategies to Mitigate the PCR Inhibitory Compounds Found in Carpet Backing

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After attending this presentation, attendees will have a better understanding of the possible benefits of potential approaches to processing carpet samples for DNA analysis in order to avoid the effects of polymerase chain reaction (PCR) inhibitory compounds found in the backing layers of interior carpet.

This presentation will impact the forensic science community by providing experimental evidence indicating that the PCR inhibitory compounds in carpet backing are concentrated in different layers of the carpet. Results will also be presented that show that by only using a portion of the backing layer, the effects of the inhibitors can be mitigated or eliminated entirely. This study may suggest potential changes to extraction protocols that laboratories may implement in order to increase

the quality of the DNA profiles of samples collected from interior carpeting.

A common feature of most residential homes, as well as many commercial and public structures, is carpeting. Evidentiary biological samples such as blood and semen are often collected from crime scenes in the form of stains soaked into this interior carpet. Highquality DNA profiles can be challenging to develop from these samples due to the presence of PCR inhibitors that interfere with the amplification process. In this study, 40 interior carpet samples were collected from different manufacturers and commercial vendors and assessed for PCR inhibition. The carpet was broken down into its four basic components: the fibers, the first backing layer, the binding/adhesive layer, and the second backing layer. Each component was examined individually and the backing layers were examined together in all combinations in order to identify if the PCR inhibitors were localized or were contained in more than one component of the carpet structure. None of the carpet fibers demonstrated any significant levels of PCR inhibition; however, 65% (26/40) of the intact carpet backing layers demonstrated significant to total inhibition of PCR product as measured with the Applied Biosystems Quantifiler® human DNA kit. When separated into individual layers, or in combinations of two out of the possible three layers, PCR inhibition was most often apparent with the first backing layer and the binding/adhesive layer. The second backing layer demonstrated little, if any, inhibitory effects on PCR. These results were further supported when the samples were amplified using the Promega PowerPlex® 16 HS short tandem repeat kit. Samples extracted from the first backing and the binding/adhesive layers demonstrated a reduced or absent STR signal, while samples extracted from the second backing layer were rarely affected. Data were collected showing that the PCR inhibiting compounds co-extract with the DNA; extracts of DNA-free carpet were not themselves inhibitory. Diluting the inhibited samples was effective at restoring signal in some instances, but not in all of them, indicating that a more efficient strategy to process samples would be to avoid the inhibitory agents to the greatest extent possible. Dilutions can also involve multiple trials, wasting sample that may already be in low quantity.

In summary, two of the three layers that constitute the backing layer of interior carpeting are indicated as containing the compounds responsible for the PCR inhibition observed when working with biological samples extracted from carpet. The carpet fibers and the second backing layer do not demonstrate significant levels of inhibition. While forensic DNA examiners could ideally avoid any PCR inhibition by only processing the fiber layer, in a sample of low quality and/or quantity, isolating every possible bit of DNA may be necessary to ensure the best possible profile. Additionally, the fiber layer is the part of the carpet most often cleaned in an attempt to destroy evidence, and therefore is not always available. In these cases, examiners will need to use the carpet backing; however, this study indicates that only the second backing layer should be used in order to avoid the PCR inhibitory effects of the two other layers.

DNA, Carpet, PCR Inhibitors

A55 STR Profiling of Bloodstained T-Shirts Submerged in a Freshwater Lake

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After attending this presentation, attendees will have a greater understanding of how bloodstains are affected by submersion in freshwater environments for variable periods of time.

This presentation will impact the forensic science community by providing data relevant to the effects water submersion has on the appearance, DNA quantities, and STR profiling of bloodstains submerged in freshwater environments for variable periods of time.

The success of DNA analysis is affected by the environment and substrate from which the sample is recovered. The rate of DNA degradation depends on light exposure, water content, temperature, and the presence of microorganisms that may result in physical, chemical, and biological degradation of the genomic DNA. Extreme environments, such as submersion in water, can greatly reduce the likelihood of successful short tandem repeat (STR) genotyping. Submerged samples are exposed to the contents of the water, which may introduce inhibitors, or degrade the bloodstain itself. Another factor acting on the bloodstain is the mechanical action of water removing cellular material from the substrate, washing away valuable evidence. To date, some research has focused on developing methodology for extracting and typing DNA from corpses, tissue, and bone that have been immersed in water; however, little has been done to explore the possibility of testing bloodstained clothing recovered from an aquatic environment. Bloodstains found on submerged clothing may offer investigators information regarding the identity of the victim and foreign DNA that may belong to a suspect or witness to the crime.

DNA extraction and DNA typing of bloodstains immersed in water have not been extensively studied. It can be envisioned that in certain circumstances where a body is absent and only circumstantial evidence, such as bloodstained clothing, may be the investigators' only piece of biological evidence. Dried bloodstains have been extensively examined for successful extraction and typing. However, these same studies have not been performed on bloodstains following submersion in water.

The goal of this study is to determine the effect water submersion has on the recovery and amplification of DNA from bloodstained T-shirts submerged in a freshwater lake for periods ranging from two minutes to 90 days. Based on the success of previous research on submerged samples, this study evaluated and compared two extraction methods, a phenol-chloroform method and the manufacturer's protocol for a commercial, silica-column-based kit. This study will serve as a baseline, utilizing whole blood and a cotton substrate to minimize degradation and possible inhibitors other than what is introduced by the aquatic environment.

All bloodstains recovered from the lake, deionized water controls, positive controls, and negative controls were divided and extracted using two methods: a commercial, silica-column-based kit, and an organic extraction method. DNA was quantified using real-time PCR, and STR profiles were generated using a commercial multiplex kit.

Preliminary results suggest that bloodstains submerged for up to 24 hours are viable for obtaining quantifiable amounts of DNA capable of producing full Short Tandem Repeat (STR) genotypes. Findings have also shown that the appearance of the bloodstain following recovery from the lake does not necessarily correlate to the success of DNA analysis. The preliminary results from bloodstains submerged in deionized water indicate greater quantities of DNA from the same time periods, suggesting that either the water currents or contents impact the amount of DNA retained by the substrate.

DNA Analysis, Bloodstains, Submerged

A56 Validation of an Alternative for DNA Extract Purification Using the QIAamp® DNA Mini Kit in mtDNA Analysis

Ashley M. Collins, MS, Constance L. Fisher, PhD, and Erica M. Ames, MS, FBI, 2501 Investigation Pkwy, Quantico, VA 22135*

After attending this presentation, attendees will learn that the QIAamp DNA mini kit is a viable alternative for extract purification from hairs, blood, and buccal samples for mtDNA analysis.

This presentation will impact the forensic scientific community by providing a study that compares extraction methods through mtDNA amplification yields.

The purpose of this study was to determine if an alternative method for DNA extract purification using the QIAamp® DNA Mini Kit is comparable to the current method of Phenol Chloroform Isoamyl Alcohol (PCIA) purification for hair, blood, and buccal samples. Side-by-side comparison of the QIAamp® purification with the PCIA purification

method was assessed by yield of amplified mitochondrial DNA, overall sequence quality, and contamination susceptibility.

To test DNA extraction from hairs, 12 hairs from 10 different individuals of known sequence were extracted and purified with both methods (exception: 2 hairs from extensions made from human hair were not previously sequenced). Hair cuttings effectively represented samples of 2 cm, 1.5 cm, 1 cm, and 0.5 cm in length. Twice these lengths were initially cut (no root material was used), ground in a microtissue grinder in Stain Extraction Buffer (SEB), and incubated at 56°C for two hours to overnight. The extract volume was split into two—one purified with PCIA and the other purified using the manufacturer's instructions for the QIAamp® DNA Mini Kit. The hair samples were amplified for two subregions of HV1 and HV2: "HV1b" spanning positions 16160-16390 and "HV2b" spanning positions 178-408 using 36 cycles. Post-amplification yields were measured using the DNA 1000 assay on the Agilent Technologies 2100 Bioanalyzer instrument. Cycle sequencing was performed using the Big Dye Terminator v.1.1 Cycle Sequencing kit. Post-cycle sequencing clean up was performed using the CentriSep™ columns and then run on a 3130xl Genetic Analyzer capillary electrophoresis instrument for visualization of the sequence products.

In addition, 10 total blood and buccal samples from different individuals of known sequence were extracted and purified with both methods using a similar experimental design to the one described above. For blood samples, approximately 1.5mm^2 cuttings were taken, and ¼ of a swab was cut for buccal samples. The DNA extracts from the blood and buccal samples were amplified for the Whole Control Region (WCR, spanning nucleotide positions 15998-616) using 32 cycles. Post-amplification yields were measured using the DNA 7500 assay on the Agilent Technologies 2100 Bioanalyzer instrument.

There was no effective difference in the yield of amplified mitochondrial DNA based on length of starting material for the hairs between the extracts purified with the different methods. In fact, no overall pattern emerged in the data to indicate one method consistently provided a higher yield over the other for hairs, blood, or buccal samples. A vast majority of the samples exhibited little to no difference in the amount of amplified mitochondrial DNA when comparing the two procedures. One exception was a single observation of a threefold higher difference in amplified mitochondrial DNA yield for one bloodstain sample purified with PCIA. However, the extract from the corresponding QIAamp® purification for that bloodstain appears to have been inhibited, since dilution of the extract was required for successful amplification. It is also important to note that two other bloodstains had a 1.7-1.9 fold higher yield with QIAamp®. Both these observations, taken with the fact that all yields for the blood samples were relatively low, indicate caution is necessary when making the decision as to which treatment to use when processing bloodstains. There was virtually no difference in the sequence quality generated or the number of length variants detected between the two purification methods. The results also indicate little difference between the two methods with respect to the incidence of contamination, despite the higher number of manual manipulations with the QIAamp® columns. Therefore, using the QIAamp® DNA Mini Kit for purification for hair, blood, and buccal samples is a viable alternative to PCIA purification.

Extraction, mtDNA, Comparison

A57 Evaluation of Commercial Kits for Co-Extraction of RNA and DNA From Human Body Fluids

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After attending this presentation, attendees will learn that for situations for which both RNA and DNA are desired from the same evidentiary sample, valuable material and time can be saved by simultaneously processing the sample using various commercial kits.

This presentation will impact the forensic science community by providing information that may aid forensic scientists in making a decision as to which commercial kit to use for the dual extraction of RNA and DNA. Attributing a stain recovered from a crime scene to a particular anatomical tissue may aid in predicting the success of DNA profiling efforts, as well as helping to reconstruct the crime. RNA is transcribed in a tissue-specific manner and, thus, the reverse transcription of RNA and subsequent amplification of tissue-specific markers have been developed to supplement more conventional methods of body fluid identification. The simultaneous extraction of both RNA and DNA from the same stain is desirable in order to use the least amount of evidence in the analysis.

The purpose of this study was to evaluate several dual extraction methods for their ability to provide DNA and RNA of sufficient quality and quantity for successful downstream applications. Blood and saliva samples were collected from 30 donors. A preliminary study was performed with blood spotted onto cotton swabs for 10 donors. In order to establish a reference to which the dual extraction kits could be compared, samples were purified with traditional DNA and RNA extraction methods (i.e., organic extraction, Ambion® RNAqueous®, and TRIzol® Plus). The dried spots were co-extracted using the Zymo Research ZR-Duet™ DNA/RNA MiniPrep kit, the Qiagen® AllPrep DNA/RNA Mini kit, the Norgen Biotek RNA/DNA/Protein Purification Plus kit, and the Fisher SurePrep™ RNA/DNA/Protein Purification kit. Purified RNA was reverse transcribed to cDNA. Both DNA and cDNA were quantified by real-time PCR. Amplification of cDNA was carried out using primers for fluid-specific markers and purified DNA was amplified using primers for STR markers. All amplicons were detected on a capillary electrophoresis instrument.

Quantification of the DNA extracts indicated that the highest average concentrations were observed with the Zymo and Qiagen kits (0.828 and 0.318 ng/uL, respectively). The lowest average concentrations were observed with the Norgen and Fisher kits (0.013 and 0.007 ng/uL, respectively). The peak heights and the frequency of artifacts or aberrations such as allele dropout were used to assess the quality of the STR profiles generated from co-extracted DNA.

The ability of each kit to yield RNA extracts free of genomic DNA contamination without relying on optional DNase treatments was examined. Laboratories that analyze nuclear DNA are unlikely to allow the incorporation of DNase in the workflow. The detection of a housekeeping gene (beta-2-microglobulin) and a blood-specific marker (erythroid delta-aminolevulinate synthase) were used to assess the ability of co-extracted RNA to yield successful identification of the body fluid source tissue.

The results of the preliminary study were used to select the best of the four kits for further analysis of both blood and saliva samples from the remaining 20 donors. In addition to assessing extraction efficiencies and the quality of genetic profiles generated, the co-extraction methods were evaluated for cost, user-supplied reagents, sample processing time, hazardous waste generation, and required bench skills. Stand-alone DNA or RNA purification methods generally offer better extraction efficiency, but co-extraction methods may still provide a complete genetic profile while simultaneously identifying the tissue source of the stain from which a profile has originated.

RNA and DNA, Co-Extraction, Body Fluid Profiling

A58 Comparative Evaluation of Methods to Remove Exogenous DNA From Tooth Samples

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After attending this presentation, attendees will have an understanding of the development of a decontamination method that is effective for the removal of nanogram quantities of exogenous DNA from tooth samples.

This presentation will impact the forensic science community by demonstrating the effectiveness of a decontamination method that will remove amounts of exogenous DNA from a tooth expected from normal handling by examiners not protecting the samples from contamination. Forensic laboratories will be able to adopt this method to prevent contamination of these types of samples.

This presentation will summarize the evaluation of several methods for the removal of exogenous DNA deposited during previous examination, handling, or comingling of forensic tooth samples. All treatments for tooth and bone decontamination, including the original Harris County Institute of Forensic Sciences (HCIFS) method, were tested by adding and attempting to remove increasing amounts of DNA from teeth. An optimal method for DNA removal was identified and subsequently validated for use on forensic casework tooth and bone samples submitted to the HCIFS laboratory.

DNA extracted from tooth samples can provide powerful information in both forensic casework and missing person identification. The presence of surface DNA contamination from previous examinations or comingling can provide misleading or confusing results, however. This study approached systematically the development of a method to effectively remove surfacecontaminating DNA by assessing a variety of removal methods in various combinations. These techniques included prolonged soaking in bleach and/or deionized water, rinsing with deionized water and/or ethanol, and exposure to UV irradiation.

Sixteen combinations of the techniques were tested for their ability to remove five levels of exogenous DNA, at 5ng, 10ng, 25ng, 50ng, and 100ng. One combination, treatment "M," comprised of 10 minutes of exposure to 5% bleach, to deionized water, and to UV irradiation at 120,000 µJoules/cm^2 followed by a physical scrub and an ethanol rinse was the most effective method. Treatment M removed all but 0.4% of the highest level of exogenous DNA, a 36-fold improvement from the original HCIFS method.

Using the improved decontamination method M, results were reproducible, with no exogenous DNA detected for eight of the ten animal tooth-human saliva samples tested. Two samples, dosed with 25ng and 50ng amounts of exogenous DNA, yielded DNA residual amounts of 0.04ng and 0.16ng, respectively. For those two samples, 4.0% and 6.0% of the donor profiles were observed, on average, with standard deviations of 0.14 and 0.04, respectively. All other samples, with donor DNA amounts ranging from 0.1 to 22.4ng of DNA, yielded negative amplification results (that is, no alleles detected). Six of the ten samples decontaminated using the original HCIFS method yielded sufficient exogenous DNA to produce full STR profiles.

All non-probative human tooth samples cleaned using treatment M produced single-source profiles from the tooth donors only; no exogenous DNA was detected. Using the original HCIFS method, one tooth, a molar with 1µL of human saliva containing 22.4ng of DNA added, yielded a mixture of DNA from the exogenous DNA source and the tooth donor. These results indicate that treatment M is a more effective method for decontamination of a tooth than the original HCIFS procedure, removing substantially more exogenous DNA than all other methods tested. The procedure known as treatment M, a multi-step process removing exogenous DNA, may be of general utility of cleaning teeth prior to analysis.

Tooth, Bone, Decontamination

A59 Internal Validation of the AmpFℓSTR® Identifiler® Plus PCR Amplification Kit and Comparison to Identifiler® for the Boston Police Department Crime Laboratory

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After attending this presentation, attendees will be well-informed of the performance of the Identifiler® Plus amplification chemistry on reference and mock casework samples as compared to the Identifiler® amplification chemistry.

This presentation will impact the forensic community by comparing and contrasting the performance of Identifiler® versus Identifiler® Plus, and discussing the improvements and limitations of Identifiler® Plus so DNA laboratories may determine which amplification chemistry is best suited for their individual needs.

Amplification is an important step in the process of generating a DNA profile, allowing as little as a few cells to generate millions to billions of copies so that the sample DNA can be detected. The amplification kit selected can affect the quantity and quality of results obtained. Therefore, great care should be taken in choosing the DNA amplification kit that is best suited for the needs of the laboratory. The Identifiler® Plus amplification chemistry is an improvement over the first generation Identifiler® amplification kit. While both kits amplify the same loci with the same primer sequences in the same concentrations, the Identifiler® Plus amplification kit has demonstrated improved performance on severely inhibited samples, greater sensitivity, improved performance on mixture samples, a cleaner baseline, and a reduced number of artifacts due to an improved primer manufacturing process. The Identifiler® Plus amplification kit is able to amplify a lower target amount with the same or better sensitivity as the Identifiler® amplification kit. The Identifiler® Plus amplification time is also one hour shorter than Identifiler® time.

An internal validation was performed for the Identifiler® Plus amplification kit at the Boston Police Department Crime Laboratory. The results of the validation support a protocol of amplification for 28 PCR cycles with a DNA target input of 0.75ng and an injection time of 10 seconds on a 3130xl genetic analyzer. The validation studies confirmed the precision stated by the manufacturer for the kit and showed full concordance in allele calls with the Identifiler® amplification kit. The sensitivity studies showed that full singlesource profiles above the analytical threshold could be obtained from a target input of 0.125ng, but not all peaks were above the stochastic threshold at that target. Reproducibility in allele calls and peak heights was also seen in samples that were amplified two weeks prior. Mixture studies demonstrated that the Identifiler® Plus amplification kit was able to detect a mixture sample when the minor component was present as 5% of the total DNA and that a full profile from the minor component was often generated when present as 25% of the mixture sample. A full profile for the minor component was rarely obtained when the minor component was present as 10% of the total mixture. No contamination was found in the negative controls throughout the validation studies.

The Identifiler® Plus internal validation instituted a double threshold for analysis, with an analytical and stochastic threshold put in place. This varied greatly from the single calling threshold that was in place with the Identifiler® amplification kit. Amplification with Identifiler® Plus on touch DNA and degraded samples provided more allele calls than when the samples were amplified with Identifiler®, due to both the increased sensitivity and performance of the kit, as well as the lower analytical threshold in place for calling. However, many alleles that had previously been able to be used for statistical purposes in the Identifiler® amplification samples were now falling between the analytical and stochastic threshold for the Identifiler® Plus samples. These alleles were no longer able to be used in statistics and could be used for exclusions only. Nevertheless, the Identifiler® Plus amplification kit provided more

information overall and should be considered for use over the Identifiler® amplification kit.

Identifiler® Plus, Validation, Comparison

A60 An In-Depth Look: 412 Property Crime Cases From the Low-Country Region of South Carolina

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After attending this presentation, attendees will understand what constitutes a property crime according to South Carolina legislation and will be introduced to potential patterns and trends noted in this study of 412 cases and 879 questioned samples. Questioned samples and sample types will be explored as to which resulted in profiles and/or CODIS hits.

This presentation will impact the forensic science community by highlighting the questioned samples and sample types that produced DNA profiles. The types of samples analyzed (blood, saliva, and touch) as well as whether the crime was against a residence, public building/business, person, or vehicle will be discussed.

The low-country region is a geographical region that includes Charleston, South Carolina and the surrounding counties. The four agencies that submitted cases for this project included: the Mount Pleasant Police Department, the Charleston Police Department, the Charleston County Sheriff's Office, and the North Charleston Police Department.

According to South Carolina Legislature, Chapter 11, Offenses against Property, a property crime includes arson, burglary, housebreaking, robbery, and robbery of a person operating a motor vehicle. The distinction between burglary and breaking and entering will also be noted.

In total, 879 questioned samples were processed and analyzed as part of this project. Of the questioned samples 378 were blood, 151 were saliva, and the remaining 350 samples belonged to the category "touch." A brief description of items in this category will be explained. Of the blood samples 94% produced DNA results, additionaly, 64% of the saliva samples and 25% of the touch samples resulted in DNA results.

Of the cases submitted, 36% of the cases were crimes against a residence, 30% of the cases were crimes involving a public building or business, and 33% of the cases were against a vehicle. Less than one percent of the cases submitted were committed against a person. Although all three major categories of crimes had roughly the same number of cases, the types of samples collected (blood, saliva, and touch) differed greatly. Those samples that produced a DNA profile and/or a CODIS hit will be discussed and compared in each of the three categories.

The results were surprisingly variable. One may anticipate that similar percentages of blood, saliva, and touch samples would be collected at a crime against a residence, public building/business, person, and vehicle, but that does not appear to be the case. Hypotheses will be discussed as to why those differences may have occurred.

This presentation will be followed by a comparison presentation regarding the property crimes cases analyzed from different states. In total, the project will involve three locations and over 1,800 cases. A snapshot of an additional future study will also be noted in this poster. It will briefly address what percentage of each case resulted in a conclusion in the adjudication process.

The trends noted across these 412 cases will be presented. It is recommended that persons in the field, especially individuals involved with the collection of evidence and those involved in DNA analysis should become familiar with the patterns visible in this study.

Property Crime, South Carolina, DNA

A61 Multivariate Statistical Evaluation of Bacterial rRNA16S V4-V6 Sequencing to Identify Soil Evidence

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After attending this presentation, attendees will understand the forensic identification of soil samples based on next-generation sequencing and multivariate statistical analysis of 16S ribosomal RNA bacterial sequences.

This presentation will impact the forensic science community by introducing a novel molecular approach to soil identification. The advantages of assaying bacterial 16S rRNA loci compared to traditional physical and chemical evaluation of soil samples will be illustrated with an emphasis on the implementation of statistical methods for mathematically confident soil identifications.

Forensic soil analysis has historically been conducted through physical and chemical examination, including color determination, particle size distribution, and chemical component analysis. While these procedures have met with some success, there are a number of shortcomings, including lengthy preparation times, the amount of sample available for testing, subjectivity of results, and inconclusive data. Development of methods that reliably and statistically show soil origination is imperative.

Over the last eight years, forensic biologists at Michigan State University have been examining various molecular methods for characterizing and identifying soil samples based on their microbial populations. Early investigations using Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis showed that bacterial communities tend to differ among soils but often produce extremely complicated profiles, making reproducibility difficult. Further, these methods do not allow statistical analyses. Quantitative Polymerase Chain Reaction (qPCR) was then employed to assess if proportional comparisons of specific bacterial populations could differentiate soils. Pairwise comparisons of soil samples showed potential for differentiating them; however, not all soils could be completely separated.

With recent advances in technology, sequencing large quantities of DNA is both feasible and relatively inexpensive, making it a viable option for forensic utilization. In this study, soil samples from a wood lot, a marsh, and a yard were collected over time, and DNA was isolated. Using barcoded universal bacterial 16S rRNA primers, which allow for multiple samples to be sequenced and differentiated bioinformatically, the V4–V6 regions were amplified and sequenced using high-throughput pyrosequencing. Sequence files were processed using the software "mothur," where barcodes and sequences too short or with ambiguous base calls were removed, and informative sequences aligned. A square phylip-formatted distance matrix was produced showing the pairwise distances between each sequence in the data set with a cutoff of distances greater than 0.30. Bray-Curtis and Sørensen indices were also calculated to exemplify the compositional dissimilarity between the samples. Additionally, the sequences were clustered into operational taxonomic units, which are bins composed of sequences 97% or more similar, based on the average neighbor method.

Nonmetric Multidimensional Scaling (NMDS) and Principal Component Analysis (PCA) were employed to evaluate the reproducibility of the analysis, and to examine which was more appropriate for the data set. NMDS is a numerical ordination technique that searches for a true best solution to the explicitly chosen axes. It has advantages over PCA in that it does not assume linear relationships within the data and is outside the restraint of the eigenvalue-eigenvector technique. However, NMDS is limited in that, being a numerical technique, it allows the possibility of different outcomes under the same conditions, and may not identify the true best solution based on computational limitations. PCA is an analytical ordination technique that identifies relationships based on variance. The first principal component, or axis, is plotted to include the greatest variance within the data set. A second principal component is then plotted perpendicular to the first along the axis of the second greatest variance. More principal

components can be plotted; however, three is usually sufficient to elucidate relationships and more than three becomes difficult to visualize. PCA has advantages over NMDS in that the plot produced is easily understood and clearly illustrates relationships within the data set as well as reducing subjectivity in that axes are chosen based on the linear relationships in the data.

The ability to identify unknown soil samples via these techniques was then investigated. "Questioned" samples were picked blind from the original soil plots and processed as above. Based on both NMDS and PCA plots, the questioned samples were statistically included within the appropriate soil site. Likewise, samples 10 feet from the original collection sites were tested. The methodology proved useful for both assigning the origin of soil, and giving a percent confidence of association to that group. Plots designed by the software are easy to understand and explain in court. Furthermore, the addition of statistical confidence allows the technique to be more readily accepted as a reliable identification process, and helps meet *Daubert* considerations thus, this methodology has clear utility for forensic scientists.

Soil Identification, Multivariate Stats, DNA Pyrosequencing

A62 Internal Validation of the PowerPlex® Y23 Amplification Kit for Use in Forensic Casework

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After attending this presentation, attendees will understand the added advantage for using a Y-Chromosome Short Tandem Repeat (Y-STR) amplification kit in forensic DNA testing and the ability of PowerPlex® Y23 to perform effectively in the Colorado Springs Metro Crime Laboratory.

This presentation will impact the forensic community by demonstrating the robustness, reliability, and reproducibility of PowerPlex® Y23 in a forensic DNA laboratory setting through comparison with other commercially available Y-STR amplification kits and full validation.

Over the past decade, forensic laboratories have come to rely on commercially available Y-STR kits as a fundamental tool in forensic DNA casework where the identification of male specific DNA is essential. In an admixed sample, foreign DNA from a male is often masked by high levels of female DNA. Autosomal STR (short tandem repeat) analysis in admixtures often results in a DNA profile with major female alleles and minor male alleles. The minor male alleles are either difficult to distinguish from stutter peaks in the mixed profile or absent due to preferential amplification of the female DNA. However, the male-specific nature of Y-STR analysis allows for targeted amplification of male DNA with no amplification of female DNA, even when present in the mixture at high levels. Additionally, Y-STR analysis is advantageous in sexual assault cases involving multiple male assailants where autosomal STR analysis is ambiguous regarding the number of contributors to the mixture. Furthermore, Y-STR analysis is valuable in cases where mixed gender DNA cannot be segregated by differential extraction, such as evidence from azoospermic or vasectomized males and blood-blood or blood-saliva mixtures.

The initial stage of this study focused on the comparison of three commercially available Y-STR kits: the eleven loci PowerPlex® Y kit (Promega Corporation), the sixteen loci Yfiler™ kit (Applied Biosystems-Life Technologies), and the twenty-three loci PowerPlex® Y23 kit (Promega Corporation). Precision, sensitivity, and mixture interpretation capabilities were compared for the purpose of identifying the optimal kit for implementation at the Colorado Springs Metro Crime Laboratory. In these comparisons, all three kits demonstrated comparable performance in precision and mixture interpretation. On the other hand, PowerPlex® Y exhibited superior sensitivity over Yfiler™ and PowerPlex® Y23, with less allelic drop out at DNA target concentrations below 0.125ng/µL. Despite an increase in allelic drop out at low concentrations, however, Yfiler™ and PowerPlex® Y23 exhibited an increase in the number of allele calls overall compared to PowerPlex® Y at the same concentrations. PowerPlex® Y23 provided significantly more allele calls for all concentrations tested and was therefore chosen for full internal validation.

During internal validation, PowerPlex® Y23 was examined for precision, sensitivity, mixture interpretation, stutter, analytical and stochastic thresholds, reproducibility, concordance, and contamination. Precision of the 3130 AB Genetic Analyzer using PowerPlex® Y23 was evaluated using an allelic ladder. The average standard deviation for each allele at all loci was calculated. All loci as well as all alleles within each locus exhibited an average standard deviation value less than 0.15, indicating compliance with the 95% confidence interval. Sensitivity data was collected for DNA target concentrations of 0.0312, 0.0625, 0.125, 0.25, 0.5, 1.0, and 2.0ng/µL with amplicon loads of 1, 2, and 3µL at injection times of two, five, and ten seconds. For standard run parameters of 1µL amplicon load and a five second injection, the target DNA concentration range capable of producing a full profile was 0.125 to 2.0ng/µL. However, the recommended target DNA concentration range is 0.5 to 1. 0ng/µL. Increasing the amplicon load resulted in little success in generating additional allele calls where allelic drop-out had occurred. However, increasing injection time generally increased the number of allele calls, but did not result in a full profile at concentrations lower than 0.125 ng/µL. Decreasing the injection time from five to two seconds successfully decreased pull-up and extraneous artifacts in overloaded samples. Previously extracted non-probative samples as well as DNA standards were used to determine reproducibility and concordance. Since the Colorado Springs Metro Crime Laboratory does not currently perform Y-STR typing, each sample was amplified using Yfiler™ and PowerPlex® Y23. All samples produced reproducible results, and all alleles shared between Yfiler™ and PowerPlex® Y23 kits exhibited 100% concordance.

Y-STR, Forensic DNA Testing, PowerPlex® Y23

A63 A Study of Recombination Between 15 X-Chromosomal Short-Tandem Repeat Markers in Multigenerational Family Pedigrees

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After attending this presentation, attendees will have a better understanding of the considerations involved in utilizing multiple Short Tandem Repeat (STR) markers located on the X chromosome after viewing the results of an NIJ-funded study into the recombination rate of 15 X chromosomal STR (X STR) markers that have previously been shown to be highly polymorphic in United States populations.

This presentation will impact the forensic science community by providing an understanding of how the organization of 15 X STRs across the chromosome affects their practical application in the forensic laboratory while increasing awareness of their potential utility. In addition, this presentation will describe one of the hurdles that must still be addressed for this marker system prior to routine use, prompting other scientists and laboratories to contribute to the accumulation of this important foundational data.

X-chromosomal STR (X STR) markers have recently been recognized as useful tools to supplement traditional kinship testing in the forensic setting. Development of assays allowing the multiplex detection and analysis of various combinations of X STRs has spawned numerous publications reporting the standardization of repeat structure and distribution of allele frequencies in a number of populations across the globe. However, far fewer studies have been published exploring the

practical implications of utilizing markers located on a single chromosome.

According to the 1991 report of the International Society for Forensic Genetics (ISFG; formerly ISFH (Hameogenetics)) relating to the use of DNA polymorphisms in paternity testing, questions of independent assortment must be addressed for any forensic marker system. For autosomal STRs, this ensures that the product rule can be used to multiply individual marker frequencies together to determine the overall rarity of a profile. It does not preclude the use of linked markers, however, Y chromosomal STRs, for example, are linked to one another and are considered together as a group called a haplotype. Haplotype frequencies are measured directly from population data, and the counting method is used to determine the rarity of the profile. It follows that X chromosomal STRs may require a combination of the two techniques: the organization of several physically close markers into linkage groups, forming haplotypes, whose frequencies could then be multiplied together once independent assortment of the groups or "blocks" was established. Early linkage studies produced a map of the X chromosome that divided 16 X chromosomal STRs into four linkage groups and most subsequent studies have employed this model when considering markers to include in novel multiplexes as well as a currently available commercial X STR kit.

At the Armed Forces DNA Identification Laboratory, two multiplexes consisting of a total of 15 X STR markers (DXS 89, DXS9902, DXS7132, DXS7130, DXS6795, DXS10147, DXS8378, DXS7423, HPRTB, DXS101, DXS7424, GATA31E08, GATA172D05, GATA165B12, and DXS6803) have been characterized and allele frequencies determined for several different populations. In order to evaluate the organization of these markers into the four proposed linkage groups, 58 families (832 individuals) satisfying the requirements of linkage study (multiple generations and offspring) have been acquired and investigated. In this presentation, the results of this NIJ-funded study, demonstrating recombination between markers within the same proposed linkage group as well as confirming a mutation rate for X STRs on the order of 10^{-4} will be presented. These results confirm the hypothesis that for these 15 markers, recombination is not a negligible factor in the statistical interpretation of an association between individual and/or family profiles.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense, the United States Department of the Army, or the National Institute of Justice.

Recombination, Linkage, X STRs

A64 Cell Surface Hydrophobicity of Biothreat Agents: A Novel Forensic Signature for the Attribution of Microbial Biocrimes

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After attending this presentation, attendees will understand the significance of the growth medium composition on bacterial signatures, the analytical considerations for microbial hydrophobicity assays, and the forensic issues associated with microbial threat agent identification and characterization.

This presentation will impact the forensic science community by introducing a new technique that can assist future biocrime investigations. Because an organism's hydrophobicity phenotype is influenced by taxonomy and the laboratory conditions used for culturing, analyzing these signatures may help provide leads and/or exclude suspects during a forensic investigation.

The Amerithrax investigation in 2001 highlighted the need for forensic signatures that can rapidly identify a threat agent and determine the laboratory in which it was cultured. Since 2001, many methods have been developed to identify the species or strain of organisms in evidence recovered from a biocrime; however, few forensic signatures exist that indicate the culturing conditions of a threat agent and, therefore, the lab of origin. The few signatures that do exist require significant amounts of sample (~$1x10^6$ cells) that is often prohibitive in a forensic investigation. Most strikingly, there are no analytical techniques that simultaneously provide both types of information.

One promising strategy for addressing this need is analyzing an organism's cell surface hydrophobicity. The surface hydrophobicity of microorganisms is influenced by the proteins, lipids, and carbohydrates present on the membrane. The presence of these extracellular substances is affected by the nutrients in the growth medium and varies between culturing environments (i.e., different laboratories). Procedures for measuring bacterial hydrophobicity are well documented and include microbial adhesion to hydrocarbons (MATH) and hydrophobic interaction chromatography (HIC). These techniques are rapid (<10min per sample), relatively inexpensive, and simple to execute. Despite these advantages, MATH and HIC have never been applied biothreat agents in a forensic context.

The goal of this study was to test whether the cell surface hydrophobicity could be used to determine the species/strain of an organism, the cell type (vegetative or spore cell), and the growth conditions used to cultivate an unknown organism (*e.g.,* peptone- or tryptone-containing medium). The following organisms were used for all experiments: *Bacillus cereus* T-strain (*BcT*) vegetative cells, *BcT* spores, and *E.coli*. The cell surface hydrophobicity of each sample was measured using MATH and HIC techniques. For the MATH assay, spectrophotometric absorbance was measured for the initial and final cell concentrations of the bacteria suspended in 1xPBS that had been mixed with equal volumes of hexadecane. The HIC assay involved applying the bacterial suspension (in dH_2O) to a HP Ion Exchange column and measuring the proportion of microbes adhering in the column.

Results from the MATH assay showed that *E.coli* has a higher surface hydrophobicity than *BcT* vegetative cells (30% and 16% adhesion, respectively). In addition, *BcT* spores had a higher affinity for hexadecane than *BcT* vegetative cells (57% and 16% adhesion, respectively). *BcT* spores grown in six different medium formulations showed little variation in hydrophobicity with all samples ranging between 60% and 70% adhesion to hexadecane. ANOVA analysis of the MATH data confirmed that there were no significant differences in hydrophobicity across the six growth media types.

Results from the HIC assay also showed *Escherichia coli* and *Bacillus cereus* vegetative cells had different levels of hydrophobicity, 25% and 50% retention in the column, respectively. In addition, *BcT* spores had an affinity for the HP Ion Exchange approximately 20% higher than *BcT* vegetative cells (51% and 61% retention, respectively). An increase of ionic concentration of the suspension increased the affinity of the *BcT* spores by approximately 25%. This suggests *BcT* spores are sensitive to ionic interactions on cell surface and functionalized substrates. Given that sporulation media differs in nutrient and metal availability, the HIC assay may be a good indicator of culturing conditions. Since the MATH assay was demonstrated useful for species identification and discrimination between cell types, the combination of these two hydrophobicity assays may be a powerful technique for forensic characterization of unknown organisms in a suspected biocrime.

Bacillus Cereus, Biocrime, Hydrophobicity

A65 Maximizing mtDNA Testing Potential With the Generation of High-Quality mtGenome Reference Data

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The goals of this presentation are to inform attendees of the need for complete mtGenome reference data for forensic applications, and to report an automated, high-throughput laboratory processing workflow for the generation of high-quality mtGenome haplotypes.

This presentation will impact the forensic science community by developing mtGenome reference data, which meet the highest forensic standards, and making the data publicly available via a web-based search engine to permit their use for forensic casework.

Mitochondrial DNA (mtDNA) testing in the forensic context requires appropriate, high-quality population databases for estimating the rarity of questioned haplotypes and, in turn, the strength of the mtDNA evidence. Since 2003, the Armed Forces DNA Identification Laboratory has been systematically generating mtDNA data to augment available reference population data and, ultimately, to improve the framework upon which forensic mtDNA typing is based. These data; however, and indeed all available forensic mtDNA reference databases (SWGDAM, EMPOP), only include information from the control region (CR). While this information is obviously strengthening the foundation upon which current mtDNA identification efforts are based, these data do not adequately prepare the field for the recent and rapid advancements in mtDNA typing technologies that will soon facilitate the acquisition of entire mitochondrial genome (mtGenome) information from forensic specimens. Novel assays that quickly and easily access mtDNA coding region data for increased discrimination are now available in the form of single nucleotide polymorphism assays, sequence specific oligonucleotide strips, mass spectrometry instrumentation, and next generation sequencing technologies.

Currently; however, there is a lack of appropriate, randomly-sampled and high-quality entire mtGenome reference data suitable for forensic comparisons. Thus, this funded project intends to: (1) increase the large-scale availability of high-quality entire mtDNA genome reference population data; and, (2) improve the information technology infrastructure required to access/search mtGenome data and employ them in forensic casework. The specific goals and objectives of this large-scale databasing effort are the development of 450 complete, high-quality mtGenomes spanning three U.S. population groups, and structure and query modifications to the publicly-available EMPOP database.

To assure the generation of the highest quality mtGenome profiles, a laboratory processing workflow in which nearly all pipetting steps — from initial sample placement through sequence detection — are performed robotically, and employing a rigorous data review process. Amplification of the complete mtGenome is achieved via eight overlapping fragments, with a total of 11 samples (and the appropriate negative controls) amplified per 96-well plate. Each mtGenome is then sequenced in 135 reactions, providing redundant and overlapping forward and reverse sequence coverage across the entire molecule. This optimized, highly automated protocol reduces overall data generation costs, hands-on laboratory time and — most importantly — opportunities for human error by substantially decreasing the number of manual production steps and the extent of sample reprocessing necessary to construct complete mtGenome haplotypes.[1] The data review process follows a strategy previously used for the production of high-quality mtDNA CR sequences, which includes raw data review by no fewer than three scientists, and entirely electronic data transfer with two additional profile reviews.[2] To further assure data reliability, completed mtGenome haplotypes are compared to PhyloTree to confirm phylogenetic consistency across the eight amplicons.[3] In addition, private mutations, heteroplasmies, and transversions are re-reviewed in the raw data.

The presentation will describe the application of this workflow to the development of more than 200 complete mtGenomes from anonymized blood serum samples as part of the NIJ-funded databasing effort. The workflow reliably produced high-quality data from DNA inputs down to at least 10 pg, and the majority of samples did not require any manual reprocessing to generate complete haplotypes. The efficacy of automated processing combined with a rigorous data review strategy in preventing errors with this multi-amplicon protocol was evident from the absence of problems detected at the stage of phylogenetic data evaluation. Ultimately, this project will provide the forensic community with reliable, complete mtGenome reference data and a means to access, search, and use the data in forensic casework.

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Mitochondrial DNA, Reference Data, Data Quality

A66 New Strategies to Overcoming PCR Inhibition Using Mutant Taq Polymerases and PCR Enhancers

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After attending this presentation, attendees will gain an understanding of how inhibitors found in DNA extracts such as phenol-chloroform, humic acid, collagen, and calcium affect Polymerase Chain Reaction (PCR) amplification, and whether mutant Taq DNA polymerases and PCR enhancers will aid in overcoming the inhibition to recover a DNA profile.

This presentation will impact the forensic science community by providing potential strategies to overcome PCR inhibition that may be found in low template, low quality DNA samples such as samples resulting from degraded skeletal remains. Selection of amplification strategies and downstream methods, such as the inclusion of PCR enhancers and/or new mutant Taq polymerases, will likely improve typing on crude, inhibited samples encountered in missing person, mass disaster, and cold cases.

Since its introduction in the mid-1980s, the use of DNA profiling in forensic science has revolutionized the justice system on a worldwide scale. DNA profiling is comprised of multiple steps and procedures including DNA extraction, quantification, and amplification using PCR. PCR has permitted the analysis of very low quantity, low quality DNA samples. Crime scene samples, however, are often found in very poor condition and are often mixed with extraneous materials that may co-extract with the DNA.

There are several known, commonly encountered inhibitors to PCR: calcium, collagen; humic acid; hematin; melanin; indigo dye; detergents; and, phenol-chloroform used in DNA extractions. These inhibitors may interfere with the cell lysis or capture of components necessary for DNA extraction by causing DNA degradation and/or inhibiting DNA polymerase amplification of target DNA. A review on forensic implications of PCR inhibition was recently published.

Although the causes of PCR inhibition are still not fully known, three mechanisms have been proposed. These include: (1) binding of inhibitor to Taq polymerase; (2) blocking of amplification sites due to inhibitor-template binding; and, (3) decreasing processivity due to interaction of the inhibitor with Mg^{2+} cofactors or other components of PCR.

Detection and overcoming PCR inhibition are critical challenges faced by forensic molecular biologists and many others such as microbiologists studying soil samples, molecular evolutionary biologists studying ancient remains and preserved samples, molecular ecologists studying animal excrement, molecular pathologists studying preserved and mounted specimens, and molecular archaeologists and anthropologists studying ancient human remains. Commonly utilized methods for overcoming inhibition in the forensic DNA community include: (1) diluting the samples (thereby also diluting inhibitors in the sample); (2) additional cleaning of the sample by purification; (3) including additional DNA polymerase and Bovine Serum Albumin; (4) utilizing STR multiplexes that are inhibitor resistant such as Minifiler, Identifiler Plus, and Powerplex 16HS; and, (5) adding PCR enhancers.

In this study, a mutant Taq polymerase and two different PCR enhancers were tested for their ability to overcome inhibition: Omnitaq (DNA Polymerase Technologies); PCR enhancer cocktail (DNA Polymerase Technologies references 19 and 20); and, PCRboost (Biomatrica).

Omni taq is an inhibitor-resistant Taq polymerase mutant and PCR enhancer cocktail (PEC) consists of a mixture of nonionic detergent, L-caritine, D-(+) trehalose, and heparin. PCR and STR boost are proprietary enhancers from Biomatrica, Inc. Previous tests have shown improved amplification using PCRboost from DNA samples containing indigo dye, hematin, humic acid, and phenol chloroform.

This presentation explores the amplification enhancement of OmniTaq, PEC, and PCR boost on low quantity and low quality DNA samples that contain varying amounts of inhibitors (Phoh, humic acid, collagen, and calcium). Enhancement were evaluated on replicate 1, 0.5, and 0.25 ng samples with and without inhibitors at different concentrations using qPCR (Plexxor HY) and STR multiplex typing (Identifiler, Identifiler plus, Identifiler Direct, and Powerplex 16HS).

PCR Inhibition, Mutant Taq, PCR Enhancement

A67 Development of a Scorpion-Based Multiplex qPCR Assay for Pre-Screening Mixture Detection

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After attending this presentation, attendees will gain an understanding of the use of tri-allelic SNPs in mixture identification, how tri-allelic SNPs can be incorporated into existing qPCR multiplexes using Scorpion probes, and the importance of developing a pre-screening mixture detection assay for use in forensic casework.

This presentation will impact the forensic community by providing results of attempts to develop a qPCR multiplex capable of human quantitation, PCR inhibition, and mixture detection via tri-allelic SNPs. This work presents steps toward a pre-screening mixture assay that could help alleviate the low-level mixture deconvolution and interpretation issues faced by all forensic laboratories.

Mixture analysis of genetic profiles can be challenging for even the most experienced forensic DNA analysts. The interpretation and deconvolution of mixtures is only further complicated when the sample being analyzed is categorized as touch or contact DNA because one or both contributors' peaks often fall below the analytical threshold. Unfortunately, in the current workflow of forensic laboratories, mixture detection is not possible until the final interpretation of genetic profiles. Traditionally, forensic DNA mixtures are identified by the presence of three or more STR alleles at two or more loci. This identification method is not desirable because it is the last step in the workflow. The sample may have already been processed or consumed in such a way that analysts cannot go back and increase the input of DNA to STR amplification without significant modifications to the SOP. Previously, D1S80 and tri-allelic SNPs were evaluated for their ability to identify mixtures in a qPCR assay using the Lonza FlashGel® system for detection of alleles. Non-specific priming and gel resolution of heterozygous alleles, however, were problematic using this system. The development of a new Scorpion-based, 6-dye qPCR system for pre-amplification mixture detection could help alleviate these issues and improve both the mixture deconvolution and low-level DNA difficulties faced by forensic laboratories. Early knowledge of a mixture can provide an analyst with information that could alleviate sample consumption concerns, identify whether or not amplification input should be adjusted, and decide if surface swabs from the same item should be combined. The human DNA quantitation step provides an appropriate place to incorporate such an assay because it occurs early in the workflow and is easily multiplexed. The information allows analysts to meet the optimal DNA input range for amplification in an effort to avoid interpretation issues in the final genetic profiles. A molecular marker capable of incorporation into existing quantitation multiplex kits such as the Qiagen Investigator™ Quantiplex kit are tri-allelic SNPs. Tri-allelic SNPs are small in size, which is important in degraded samples, but also polymorphic enough to identify the presence of two individuals in a sample. Successful incorporation of tri-allelic SNPs into a quantitation multiplex requires an appropriate primer, probe, and detection system. Duplex Scorpion primers will be utilized and designed for tri-allelic SNPs rs5030240 and rs4540055 so that the primer covers the polymorphic region at the 3' end. Primers are tagged with a fluorescent molecule on the 5' end with each color corresponding to a particular SNP allele. This approach, under the correct amplification conditions, will allow for preferential amplification of the primer that is complementary to the SNP compared to the mismatched primers. Mixture detection will be possible with the interpretation of amplification curves using the Rotor-Gene Q thermal cycler, which has seven color channel detection capabilities. Initially, primer sets will be created for rs5030240 and rs4540055, and will be optimized separately in single source samples using the ABI 7500 thermal cycler. Next, primers will be multiplexed into a single amplification reaction and tested in single source samples using the ABI 7500. If successful, the primer sets will then be optimized using the Rotor Gene Q and eventually incorporated into the Qiagen Investigator™ Quantiplex kit. This multiplex will then be used to identify fabricated mixture samples and results compared with detection using the ABI 3130 genetic analyzer. It is expected that fabricated mixture samples will be identified using this system and that significant progress towards a multiplex capable of human DNA quantitation, assessment of PCR inhibition, and mixture detection will be made.

Mixtures, Tri-Allelic SNPs, qPCR

A68 Comparison Study of the QIAcube® to Manual Differential Separation: Man Versus Machine

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After attending this presentation, attendees will have a better understanding of the uses of QIAGEN's QIAcube®, and may be better able to decide whether it is an appropriate instrument for use in their laboratories.

This presentation will impact the forensic science community by helping an analyst to streamline his or her workflow, as well as increasing consistency between analysts in sexual assault processing.

Sexual assault is a serious public safety concern worldwide, with the resulting caseload backlog posing significant challenges for forensic science laboratories. Each sexual assault kit is likely to contain a number of samples with female-male mixtures on which a differential extraction must be performed. Differential extraction is the process of separating sexual assault victim epithelial cells from the perpetrator

sperm cells in order to obtain an assailant profile. Unfortunately, differential extraction is a lengthy process, requiring repeated pipetting and centrifugation. Furthermore, the quality and consistency of separation may be variable between individuals. Because of the reagent cost, time, and manual work involved in working with these cases, sexual assault backlogs have unfortunately become commonplace. In an effort to identify ways to reduce these backlogs and benefit a scientist's workflow, it is worth evaluating the qualities of automated processes. This study focused on determining the utility of the QIAGEN QIAcube® for differential separation of samples, and compared it to the current manual method. The QIAcube® was originally designed to extract nucleic acids and proteins, and it is capable of centrifuging, vortexing, pipetting reagents, and extracting a supernatant from a pellet. This study evaluated the QIAcube's® abilities, and a custom protocol, to perform differential separations on up to 12 mock sexual assault samples at a time. Experiments included a cross-contamination study using mixed female blood and semen; a sensitivity study based on a 1:3 serial dilution of semen, with and without female epithelial cells present; a reproducibility study, utilizing mixed female epithelial cells and semen; as well as a matrix or mock evidence study, consisting of a mixture of female epithelial cells and semen pipetted onto different fabric types and swabs. All studies were performed by a novice student using the QIAcube®. For comparison, the sensitivity and reproducibility studies were also performed by one or more experienced analysts, using a validated manual separation and wash procedure. Each method was evaluated with respect to cost-effectiveness, time efficiency, reproducibility, and sensitivity. The QIAcube® did prove to be a very efficient way to perform differential separations, with excellent sensitivity, and superior reproducibility. There was no sign of cross-contamination between samples, even though the tubes remain open all at once in the machine. Conversely, more reagents were wasted with the automated method. Furthermore, loading the instrument proved to be difficult at first; but it was easier to train a novice on the instrument than it was to train the novice to perform manual differential extraction. The instrument may not add hours of hands-free time, with the need to prepare the reagents and set up the instrument; however, it is possible to push "Go" and walk away for about 30 minutes while the machine performs all of the centrifuging and pipetting. Lastly, the factor of general human error—for example, bumping a tube and having to re-pellet sperm cells — is eliminated from the extraction process. In conclusion, the use of the QIAcube® has the potential to help a scientist work more efficiently simply by freeing an analyst or technician from repetitious pipetting and centrifuging.

Sexual Assault, Backlog, Automation

A69 Improved Extraction Efficiency of Human Mitochondrial DNA From Hair Shafts and Its Implications for Sequencing of the Entire mtGenome From a Single Hair Fragment

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After attending this presentation, attendees will gain an understanding of a novel protocol for extraction of mitochondrial DNA (mtDNA) from hair shafts, learning how this improved extraction method, when coupled with whole-genome amplification strategies, can increase the discriminatory power of mtDNA analysis by providing sequencing data that extends to regions of the mtGenome that are not routinely analyzed in forensic science laboratories.

This presentation will impact the forensic science community by providing insight into how optimized extraction and whole genome pre-amplification methods for difficult sample types can improve the utility of mitochondrial DNA analysis using both traditional Sanger sequencing and next generation sequencing platforms.

Forensic scientists are often faced with the challenge of limited or degraded samples, where a full nuclear DNA profile may be difficult to obtain. In these instances, mitochondrial DNA (mtDNA) analysis can be particularly useful, as mtDNA is more easily recoverable from challenging sample types such as hair shafts and bone. Existing extraction protocols generally yield enough mtDNA from two centimeters of hair shaft to reliably sequence two hypervariable regions (HV1 and HV2) located in the non-coding control region of the mtGenome. However, HV1 and HV2 comprise only about 4% of the entire mtGenome. This, coupled with the fact that mtDNA is, by nature, less discriminatory than nuclear DNA, limits the current utility of mtDNA analysis. The main objective of this research is to maximize the mtDNA extracted from two centimeters of hair shaft so that more mtGenome sequence information may be obtained for comparison to a reference sample, leading to a higher discriminatory power of mtDNA analysis.

In this study, a novel method for extracting mtDNA from hair shafts is described, which combines traditional methods with two kit-based extraction methods (Qiagen® QIAamp® DNA Investigator and Applied Biosystems® PrepFiler® Forensic DNA Extraction Kits). Comparison studies were conducted as follows: 13 two-centimeter hair fragments were subjected to the traditional manual grinding/organic extraction method and 14 two-centimeter hair fragments were processed using the newly developed extraction method.[1] All purified hair extracts were quantified using a custom real-time quantitative PCR (qPCR) assay specific for human mtDNA.[2] Hair fragments processed using manual grinding/organic extraction yielded an average of 31,440 copies per extract (60µl elution volume), while those processed using the optimized method yielded an average of 270,100 copies per extract (50µl elution volume). Not only do these results indicate a consistent nine-fold increase in mtDNA concentration, but the optimized method is also less time-consuming and can be completed in approximately four hours.

Following optimized hair extraction, targeted PCR methods were used to prepare the mtDNA for traditional Sanger sequencing. Preliminary results indicate that 84.6% of the entire mtGenome can be amplified from a single hair extract using these methods. The resulting amplicons were sequenced on an Applied Biosystems® 3130*xl* Genetic Analyzer. In addition, mtDNA was enzymatically fragmented and tagged with adaptors using Nextera® XT DNA Sample Preparation Kit, and then sequenced using an Illumina® MiSeq® Personal Sequencer. Hair extracts were also subjected to whole genome amplification (WGA) using the Qiagen® RepliG® Mini Kit in an attempt to further increase the analyzable amount of mtDNA for downstream applications. While previous efforts to pre-amplify mtDNA using various WGA methods were largely unsuccessful using extracts processed with manual grinding and organic extraction, a two-fold to 16-fold increase in mtDNA concentration has routinely been achieved when using extracts processed with the optimized method. WGA pre-amplified material was also fragmented using Nextera® XT DNA Sample Preparation Kit and sequenced using an Illumina® MiSeq® Personal Sequencer. Results of all Sanger and Next Generation sequencing studies were compared to reference sequences generated using blood or buccal samples obtained from donors and the Applied Biosystems® MitoSEQr™ Resequencing System protocol and will be presented.

References:

1. Wilson MR, Polanskey DP, Butler J, DiZinno JA, Replogle J, Budowle B. Extraction, PCR amplification, and sequencing of mitochondrial DNA from human hair shafts. Biotechniques 1995:14:662–669.
2. Kavlick MF, Lawrence HS, Merritt T, Fisher C, Isenberg A, Robertson JM, Budowle B. Quantification of human mitochondrial DNA using synthesized DNA standards. J Forensic Sci 2011:56(6):1457-63

mtDNA, Extraction, Sequencing

A70 Evaluation of PowerPlex® 18D and PowerPlex® 21 With Buccal Samples Collected on Non-Treated Paper

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After attending this presentation, attendees understand how to achieve a high first pass success rate when performing direct amplification of 1.2mm or 2mm punches from buccal samples on non-treated paper with PowerPlex® 18D and PowerPlex®21.

This presentation will impact the forensic science community by demonstrating the feasibility of two new direct amplification kits for the processing of reference samples, exploring the feasibility of increasing the size of the standard punch from 1.2mm to 2mm for direct amplification.

The efficiency of reference sample processing for databasing and paternity purposes has been greatly increased by the development of direct amplification systems. DNA samples can be collected and stored on non-treated matrices, such as the Bode Buccal DNA Collector™, until sample processing is required. The Promega Corporation has recently released two direct amplification kits, PowerPlex® 18D and PowerPlex® 21 for samples collected on both treated and non-treated collection paper. This presentation will describe the studies with each amplification system to obtain optimal results from samples collected with the Bode Buccal DNA Collector ™.

Direct amplification of reference samples eliminates the need for the time-consuming extraction and quantification steps encountered in routine processing. Eliminating these steps will save time, but it can also lead to additional amplification reactions and costs as normalization does not occur. Cellular deposition varies from individuals resulting in either excessive or inadequate samples. Eating, drinking, medicine intake, health status, and non-cooperation during sample collection will all affect the number of cells deposited onto the collection paper. This variation in cellular deposition can either lead to over or under amplification if the procedure is not optimized. Optimizing the amplification parameters to account for sample variation reduces re-sampling and re-amplification, while increasing the processing efficiency of the laboratory.

Evaluation studies optimized procedures for sampling, cell lysis, thermal cycling parameters, spectral calibration, and ABI Prism® 3130 Genetic Analyzer injection parameters for both PowerPlex® 18D and PowerPlex® 21.

These optimized parameters with PowerPlex® 18D resulted in a first pass success rate of >86% for a full 25µl reaction using a single 1.2mm punch. The remaining 14% failed internal quality standards due to either over amplification or capillary electrophoresis issues (spikes, migration, or ILS problems). Complete profiles were obtained from all one hundred (n=100) samples tested without re-sampling or re-amplification.

In addition to displaying the first pass success rate for a full reaction with PowerPlex 18D, this research demonstrated the first pass success rates for 1.2mm samples (n=100) in a 12.5µl half reaction incorporating and eliminating the 20-minute lysis incubation step.

The optimized procedures for obtaining a high first pass success rate for both full reaction (25µl) and half reaction (12.5µl) will be discussed when utilizing a single 1.2mm punch with the PowerPlex® 21 amplification system. When dealing with an aged sample, or a sample with poor cellular deposition due to one of the causes previously mentioned, a 2.0mm punch may produce better results. This presentation will display results for sample processing with 2.0mm punches with PowerPlex® 18D and PowerPlex® 21, instead of the standard 1.2mm punch. Increasing the punch size can increase the chances of obtaining a complete profile using the same parameters utilized on high-quality samples.

Direct Amplification, PowerPlex® 18D/21, Half Reaction

A71 Forensic Mixture Analysis: Pre-Emptive Separation of Whole Cells With Flow Cytometry and MHC Class I Allele Tagging

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The goal of this presentation is to introduce a novel technique for the separation of complex biological mixtures of samples from multiple individuals using flow cytometry. Attendees will become familiar with Major Histocompatability Complex (MHC) Class I glycoproteins, the nature of antibody staining for cell isolation, as well as the basic principles of flow cytometry and how they will aid in the separation of complex mixtures.

This presentation will impact the forensic science community by providing an alternative approach to complex mixture analysis, one that physically separates an individual's cells from a mixture before STR amplification and analysis. This has the potential to produce multiple single source STR profiles from an evidentiary sample composed of two or more individuals in cases such as rape.

The MHC is an extensively studied region of genes that play a key role in the human immune system. It is divided into three major subgroups denoted by class I, II, and III, and has been found to be the most polymorphic region of genes yet to be identified. The forensic potential of this region lies in the multitude of alleles observed in the human population. MHC class I genes, which are the focus of this study, are referred to as the human leukocyte antigen (HLA) and are denoted by the classical HLA-A, HLA-B, and HLA-C; but may also include non-classical HLA-E, HLA-F, and HLA-G. Further, each HLA is defined by multiple subtypes, each with a unique allele grouping (e.g., HLA-A*02). MHC Class I molecules were chosen as the region of interest due to their expression on all nucleated cells. This is in contrast to more heavily researched alleles in the MHC region such as HLA-DQα, a class II molecule that lacks expression in semen. This presentation aims to describe how polymorphisms within the MHC Class I region can be used to separate individuals from an evidentiary sample using flow cytometry, a technique widely embraced by both the medical and research communities.

For this research, the goal was to take advantage of these MHC features to develop a new forensic technique that utilizes fluorescently tagged HLA antibodies specific for allele subgroups to differentiate individual contributions to a biologic mixture. Antibodies for the classical HLA alleles (ABC) were used to tag blood, semen, and epithelial cells from several different donors. Results show that the levels of MHC expression vary among all biological fluids tested. Blood, with the highest expression, resulted in 81% of all cells being separated from negative controls. Semen, with the lowest expression, produced 8.1% positive selection. It was found that flow cytometry is a fast, economical, and efficient way to separate whole cells from a mixture. It has also been shown that the prevalence of HLA molecules produces strong immuno-staining characteristics that are clearly distinguishable from controls. Initial experiments indicate that flow cytometry is a viable option for forensic mixture separation and could be quickly integrated into a forensic laboratory due to the availability of flow cytometers in nearly all major hospitals and research facilities. Physical separation of cells prior to cell lysis offers numerous advantages over trying to decipher complex electropherograms prior to SNP and STR analysis. Initial research into this technique could provide an alternative for the analysis of common biological samples.

Flow Cytometry, Biological Mixture, MHC Class I

A72 Detection of Male DNA in the Vaginal Cavity Following Digital Penetration Using Y-Chromosome Short-Tandem Repeats

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After attending this presentation, attendees will understand the value of collecting sexual assault forensic evidence (SAFE) kits from child sexual assault victims and female victims who have been digitally (finger) penetrated. Attendees will also become aware of how analysis of vaginal swabs has been optimized with application of using Y-chromosome short tandem repeats (Y-STRs) to establish a time interval in which a deoxyribonucleic acid (DNA) haplotype can be obtained from vaginal swabs following digital penetration.

This presentation will impact the forensic science community by displaying the value of collecting evidence from sexually assaulted adolescents as well as females that report digital penetration.

Analysis of sexual assault cases can be challenging if there is no report of vaginal-penile penetration, and the difficulty of analysis increases as the time interval extends between the incident and the report. Sexual assault evidence kits are not commonly collected in the majority of cases if there is only a report of digital penetration; this is due to the belief that there will not be enough DNA from the perpetrator to be detected. The value of Y-STR genotyping has been previously established in sexual assault cases where autosomal short tandem repeats (STRs) are not suitable.[1] This allows for low copy number DNA from the male perpetrator to be detected, eliminates the need for mixture interpretation, and allows for a longer window of detection.

This study has determined that male DNA can be detected from swabs collected from the vaginal cavity following digital penetration. Initial samples analyzed prior to method optimization resulted in detection of 10 of 12 alleles. The purpose of this study is to optimize the methodology used to detect male DNA from the collected vaginal swabs. The second goal is to establish a time interval in which a meaningful Y-STR haplotype can be obtained post digital penetration and determine the likelihood ratio of obtaining Y-STR haplotypes at 1, 6, 12, 24, and 72 hours post-digital penetration.

The optimization of analysis included varying parameters of existing commercial protocols. A modification of a DNA IQ™ extraction protocol was used to maximize the initial concentration of DNA extracted from the vaginal swabs. Other modifications to the analysis method included the elimination of a quantitation step prior to amplifying the DNA extract using PowerPlex® Y to amplify only male DNA. Additional optimization steps included concentration of some of the DNA from the collected samples prior to amplification. Slight alterations to the capillary electrophoresis protocols were also made to maximize detection of the male DNA.

To obtain the samples that would simulate a sexual assault kit, male-female couples were asked to abstain from sexual activities for a given period of time prior to collection. The couples participated in vaginal-digital penetration for a discrete period of five minutes. Female participants collected four swabs prior to penetration as a control, and four swabs at each of the five time intervals. The vaginal swabs were then processed using the optimized protocol from above to allow for the greatest detection of male DNA. To date, results have shown that full PowerPlex® Y profiles have been obtained from vaginal swabs collected one hour following consensual digital penetration

Reference:

1. Sibille I, Duverneuil C, de la Grandmaison GL, Guerrouache K, Teissiere F, Durigon M, *et. al.* Y-STR DNA amplification as biological evidence in sexually assaulted female victims with no cytological detection of spermatozoa. For Sci Int 2002;125(2): 212-16.

Y-STR, Sexual Assault, Digital Penetration

A73 Application of Pressure Cycling Technology (PCT) in Differential Extraction

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After attending this presentation, attendees will understand the principles behind a rapid and efficient method of extracting sexual assault evidence using a pressure-based protocol that is designed to burst open and extract DNA from male sperm cells in rape kits or other mixed forensic stains.

This presentation will impact the forensic science community by providing a better understanding of how the application of pressure pulsing can be used for more specific detection of individual cells that will enable rapid and selective processing of sexual assault evidence.

Separating the sources of DNA from different contributors to a stain reduces the difficulty associated with mixture analysis and data interpretation. The processing and interpretation of such mixed DNA samples has long been recognized as a bottleneck in forensic DNA analysis. Organic differential extraction is the most commonly used method to isolate sperm DNA from sexual assault evidence. This two-step extraction procedure involves selective digestion of epithelial cells in the first step, followed by isolation and digestion of sperm cell pellet. The major disadvantages of this technique are incomplete separation of sperm and non-sperm fractions, particularly in samples that are overwhelmed by large numbers of female epithelial cells relative to sperm cells, and the time-consuming nature of the process.

The objective of the study was development of a method using Pressure Cycling Technology (PCT) combined with reagents to selectively disrupt sperm or epithelial cells and recover DNA. The extraction procedure is performed utilizing the Barocycler® NEP 2320, a commercially available instrument from Pressure Biosciences, Inc., equipped with a hydrostatic pressure chamber that generates alternating cycles of ambient and high pressures with a range of 5 to 45 kpsi. Samples such as cotton swabs or cuttings of cloth can be directly extracted using this technique by simply placing them in a pressure cell along with an appropriate buffer.

The current study involves the application of pressure cycling technology in the selective digestion of sperm cells from evidence mixtures collected from different substrates with an emphasis on the role of buffer composition on sperm DNA yields and increase in selectivity of extraction. The cells were extracted into 1X PBS buffer (pH 7.4) with varying buffer compositions and subjected to a pressure of 45,000 psi. This pressure treatment was followed by phenol chloroform isoamyl alcohol purification to obtain a clean DNA sample devoid of salts and proteins for successful downstream analysis. The purified DNA was quantified with Promega Plexor® HY system followed by an STR analysis using Promega PowerPlex® 16 HS system.

The results indicate that selective extraction of sperm DNA is possible from mixtures in the presence of appropriate buffers. These observations were applied to different substrates and mixtures with varying ratios of sperm and epithelial cells to determine the selectivity of the extraction. The quality of the DNA recovered from pressure treatment was assessed by performing STR analysis using Promega PowerPlex® 16 HS system. Preliminary data indicate the potential of PCT application in analyzing samples from sexual assault cases, in particular, indicating improved extraction of sperm DNA at high pressures when compared to epithelial cells.

Differential Lysis, Pressure, Sexual Assault

A74 An Evaluation of MicroRNA Stability and Internal Standard Selection for Forensic Body Fluid Identification

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After attending this presentation, attendees will gain an understanding of microRNA expression in biological fluids, specifically, the advantages of microRNA analysis as a potential method of forensic body fluid identification.

This presentation will impact the forensic science community by familiarizing scientists with the concept of microRNA analysis for body fluid identification purposes, a relatively new area of research in forensic science, focusing on the stability of microRNA targets after being subjected to contamination and physical treatment conditions similar to those seen in crime scene evidence.

While forensic DNA analysis has reached a level of maturity in the forensic science field with regards to the sophistication of the techniques and confidence in the results, the equally important question of body fluid identification has lagged behind, and could still be considered to be in a primitive state. While there is widespread confidence in the DNA profile generated, there is often significantly less assurance of the identity of the body fluid from which the DNA profile was developed. It is common during trials for attorneys to categorically accept the STR analysis, but probe the forensic scientist on the source of the DNA that generated the profile. Because of this dichotomy, significant efforts have been made over the past ten years in order to develop forensic serological techniques of a more discriminatory nature.

Recently, there has been some work in the forensic science field in regards to exploring microRNAs (miRs) for a molecular-based, forensic body fluid identification method. MiRs are small structures that specifically repress protein expression through binding to messenger RNA (mRNA) in the cytosol. There are no known postprocessing modifications, and thus miRs are simpler, and potentially less problematic for detection than proteins and mRNAs. Because of their small size and lack of a poly-A tail, miRs are inherently less susceptible to degradation than mRNA. Additionally, miRs are very hardy, and have been recovered from highly compromised samples including Formalin-Fixed Paraffin Embedded (FFPE) tissue. In serum, miRs have been shown to survive harsh conditions such as boiling, low or high pH, cycles of freeze-thaw, and extended storage. Moreover, there is considerable evidence that many miRs are encapsulated in an exosome, which, depending on the microRNA and the secretion process, could be membrane-based or protein-based. Because of this, recent studies have shown that samples can even be treated with RNase enzymes and the encapsulated miRs are still detectable. This implies a high degree of stability of the species, and therefore, a very good possibility that if body-fluid specific miRs are found and described, a very robust miR method for forensic body fluid identification could be developed.

Biological evidence is naturally compromised when it is left or deposited under nonsterile conditions of a variety of environmental factors and chemical contamination. This study evaluated microRNAs under environmental and treatment conditions that forensic evidence could be subjected to. Blood, urine, semen, and saliva were collected and samples exposed to chemical treatment, prolonged high temperatures, and multiple freeze/thaw cycles. No differences were observed in microRNA levels using qRT-PCR, regardless of the number of times the sample had been frozen and thawed. Similar results were observed with other treatment methods. Detection of various internal reference controls typically used as standards (RNU6B, SNORD48, etc.) were found to vary between body fluids, which could potentially complicate studies attempting to compare relative expression levels of target miRs between biological fluids. Evaluation of additional forensically relevant body fluids, as well as more candidate internal control miRs, should be evaluated before target miRs specific for each biological fluid can be chosen and evaluated for implementation.

MicroRNA, Semen, Blood

A75 Improved Discrimination of Duct Tapes Using Carbon and Hydrogen Isotope Ratio Variations in Duct Tape Polyester Web Fibers

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After attending this presentation, attendees will understand the forensic isotopic information contained in duct tape web materials.

This presentation will impact the forensic science community by providing additional isotopic tools for in-depth duct tape investigations.

At the Netherlands Forensic Institute (NFI), Isotope Ratio Mass Spectrometry (IRMS) is used in combination with other techniques (visual, FTIR, LA-ICP-MS, etc.) to investigate potential relations between different materials in forensic investigations. A prominent example material is (gray) duct tape as it is now commonly used in Dutch households and often found at crime scenes. If a visually similar tape is retrieved during, e.g., a search at a suspects home, a request is almost always made to compare the tape materials. If tapes are not discriminated using visual comparison (color, thickness, width) and FT-IR, other techniques such as LA-ICP-MS and IRMS are used to investigate chemical and isotope characteristics.

Duct tape mainly consists of three different layers: (1) a polyethylene backing film; (2) a network of cotton or polyester fibers (web); and, (3) an adhesive layer (glue, often isoprene based). The web is a network of cotton or polyester fibers usually woven with length and crosswise threads commonly referred to as warps and wefts, respectively.

Previous work has shown that isotope ratios (d13C and d2H) of the backing film isolated from the adhesive layer can be of value in making discriminations. The value of the forensic evidence is increased by considering isotope ratios (d13C and d2H) of the backing film in isolation from the adhesive layer. The background variation of carbon and hydrogen isotope ratios from the backing was evaluated using 44 duct tape rolls of major brands randomly purchased in the Netherlands (van Breukelen *et al*., 2010).[1]

To date, the NFI gray duct tape collection comprises 120 tape rolls. Results will be presented on the discrimination of these rolls based on the backing d13C and d2H ratios.

Secondly, the added forensic value of d13C and d2H ratios for the cotton/polyester layer in duct tapes will be demonstrated. Because the isotope discrimination between different batches produced during a short production period was weak using the backing material only, an analytical procedure was developed and validated to determine d13C and d2H from the isolated web material. The warp and weft threads are found to have, in general, different d13C and d2H values; differing e.g., up to 2.5% in d13C values. This difference may be explained by the manufacturing process where presumably raw materials from different sources or batches are used for warp and weft threads, providing an additional level of discrimination. The d13C and d2H isotope variation of warp and weft bundles, as well as within individual warp and weft threads, is shown to be insignificant.

Nineteen gray duct tapes were investigated using this web method. Using the additional isotope information of the warp and weft threads, 17 of these tapes could be discriminated.

Results will also be presented from the application of this web method to representative samples from seven production rolls provided by the Dutch producer “Supertape.” The samples from these rolls of

12m length (produced between March 21, 2007 and April 12, 2007 to manufacture small tape rolls) were indistinguishable based on their backing d13C and d2H isotope ratios. It will be evaluated how much discrimination improves if the d13C and d2H isotope ratios of the polyester web are included.

Reference:

1. Discrimination of duct tapes using Isotope Ratio Mass Spectrometry, M.R. van Breukelen, F. Vogelpoel, M. Schrader, W. Wiarda, A.J.J. van Es and G.J.Q. van der Peijl, Fourth FIRMS Conference, Washington, April 12-14, 2010, book of Abstracts, p 33 (to be accessed through http://www.forensic-isotopes.org/assets/2010-Abstracts.pdf)

Duct Tape, Web, IRMS

A76 A Multichannel Microfluidic Cartridge for Rapid Forensic DNA Analysis

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After attending this presentation, attendees will understand the development and functionality of a system developed for fully-integrated microfluidic forensic DNA analysis.

This presentation will impact the forensic science community by demonstrating the advantages of using a microfluidic system for DNA analysis, including a reduction in analysis time, footprint of the microfluidic cartridge, and a reduction in consumables required for end-to-end analysis that ultimately will allow forensic scientists to process more samples.

STR typing is the accepted gold standard for human identification, and is now successfully employed in forensic, civil, and military laboratories. Although highly successful and reliable, the process typically requires 8-10 hours to complete under routine conditions, employs large sample volumes, costly reagents, and is labor-intensive. Additionally, samples are susceptible to contamination as they are exposed to the environment at multiple points during sample processing. Transitioning sample processing and analytical methods to the microscale format will permit automation, miniaturization, and integration, providing the end user a system capable of expedited, cost-effective analysis in a closed system that reduces sample handling and possible contamination.

Previously, a system capable of performing PCR and ME on a plastic chip following LE in a tube was presented. Although this system was capable of detecting 16-18 STR loci in <75 minutes, it required human intervention prior to PCR and was not capable of performing true end-to-end DNA testing.

The work presented here highlights improvements to the fully-integrated system. The transition to a plastic microfluidic cartridge for fully integrating sample preparation, PCR, and ME is described, and is the first step toward cost-effective analysis using a single-use chip with reagents on-board. With the improved system, expedited purification of DNA from crude samples is performed, and a mixture of DNA and commercially-defined PCR reagents are guided into chambers on a device for quadriplexed PCR analysis. Rapid amplification of 16-18 STR loci is achieved through use of an IR laser for non-contact heating and a non-contact method for temperature sencing. A six-fold reduction of the conventional amplification time is feasible while still achieving STR profile quality required for forensic interpretation. Simultaneous amplification of multiple samples is presented, demonstrating the capability of increased sample throughput. Following PCR, precise fluidic control allows for movement of the amplified product into the separation domain of the device. Electrophoretic separation of the amplified fragments is performed with five-color fluorescence detection using an improved detection system capable of multiplexed detection. Single-base resolution is achieved during a separation that requires <12 minutes, a three-fold time reduction from conventional separation and detection processes. A software system allows for all processes to be completed without user intervention, including automated and accurate allele calling of samples from multiple donors. An overview of the functionality of the integrated instrument capable of accepting the microfluidic cartridge will be presented, with data supporting the capability of the microfluidic system for rapid, automated, end-to-end genetic analysis for human identification.

DNA, STR, Microfluidic

A77 Development of an Integrated Microdevice for DNA Extraction and Amplification of Forensic Samples Using Infrared-Mediated Heating and Centrifugal Force

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After attending this presentation, attendees will have a better understanding of the importance of micro-total analysis systems (μTAS) for forensic DNA applications, the challenges faced with integration, and the barriers to the adaption of macroscale work to a microscale format.

This presentation will impact the forensic science community by describing a conceptual integrated DNA extraction and amplification plastic microdevice for use in PCR analysis of STR multiplexes. This work presents a step toward a μTAS that eliminates the use of expensive processing and bulky external attachments.

The value of μTAS for forensic DNA analysis lies in the potential to reduce contamination by eliminating sample transfer steps, which contribute to sample loss and reduce the acquisition of a full STR profile. Additionally, cost and lab space required for equipment, reagents, and laboratory time and effort required to perform each step in preparation for analysis is reduced. The pioneering of two key technologies, infrared (IR)-mediated heating for PCR and DNA extraction via ZyGEM chemistry, has allowed for greater possibilities in the microfluidic analysis of DNA, especially for forensic samples. IR-PCR expedites amplification with 30 cycles completed in 30 minutes followed by established, successful detection, and separation of STR loci. ZyGEM offers faster reaction times, reduced sample handling, and elimination of PCR-inhibiting reagents, while maintaining performance equal to traditional methods, making it ideal for use in an integrated microdevice. The goal of this study is to integrate DNA extraction and amplification by exploiting these technologies onto a single microdevice utilizing a centrifugal platform. Previous work has accomplished integration with the use of valve systems requiring syringes, external pumps, and other bulky, expensive attachments. However, simple valves, such as hydrophobic and one-way, single-use valves can be employed on a centrifugal platform using various speeds to effectively direct flow. In addition, no barrels or column attachments are required as the swab cutting is added directly to the microdevice, with DNA elution accomplished with the addition of reagents and spin-directed flow to an extraction chamber.

In this study, a poly (methyl methacrylate) (PMMA) microdevice was designed for ZyGEM-based DNA extraction and amplification from a buccal swab. The microdevice was manufactured in-house by laser ablation and thermal bonding. The thickness of PMMA was chosen to minimize the thermal mass of the microdevice. Heating was provided by an IR-lamp positioned below the chip. Chip design and microfluidic optimization was accomplished through a series of dye tests: blue dye

represented DNA extract while yellow represented PCR reagents. Various spin times and speeds were tested to establish the optimum parameters. The design included ZyGEM elution and extraction chambers, valves for channel sealing and metering of extract and PCR reagents, and an IR-PCR chamber. One-way, single-use "tape" valves were used to stop back flow from the extraction and PCR chambers and prevent evaporation during heating. Tape valves exploit the properties of PCR film and double-sided tape to effectively close a channel providing single-use, one-way valves for metering and channel sealing. A hydrophobic valve, for metering, connected the extract reservoir to the PCR reaction chamber. This valve "opened" once the chip was spun at a velocity such that the centrifugal force exerted on the liquid forced it through the channel. The reservoir was designed to allow for accurate volume delivery of the extract in the previously determined extract: PCR master mix ratio. PCR reagents were loaded on the microdevice and directed into the PCR chamber with the extract. Accurate metering was necessary to achieve the appropriate proportions of PCR master mix to DNA extract for successful PCR. The centrifugal platform was chosen to increase throughput (two chips must spin simultaneously to balance the system; multiplexing with four or more chips is possible) and to minimize manual interaction with the device. After establishing microfluidic control for the integrated device, ZyGEM and PCR chemistry, on-chip with IR-mediated heating, will be optimized for buccal swabs. The results of the micro-extraction and amplification will be compared to a tube-based ZyGEM extraction, followed by traditional PCR amplification via a block thermocycler.

The preliminary results suggest accurate metering can be achieved on a centrifugal platform with this newly designed integrated device. Successful integration in this system, coupled with the ease of fabrication, promotes progress towards a simple sample-in, answer-out microdevice.

Integration, Centrifugation, Microdevice

A78 Integrated Direct Amplification and Hybridization-Induced Aggregation for End-Point Detection of the Human TPOX Locus From Whole Blood

Briony C. Strachan, MSci, Hillary Sloane, BSc, Jacob C. Lee, and James P. Landers, PhD, Univ of Virginia, Dept of Chemistry, McCormick Rd, Charlottesville, VA 22904*

After attending this presentation, attendees will gain an understanding of the development of single chamber, direct amplification of whole blood, followed by label-free visual detection of the product through hybridization-induced aggregation (HIA).

This presentation will impact the forensic science community by introducing a microfluidic method that presents the possibility for the direct amplification of the TPOX locus for the rapid verification of the presence of human blood. This work demonstrates the use of a micro-total analysis system for forensic onsite analysis.

Microfluidic devices offer numerous advantages to current forensic analyses, including low sample consumption and a reduction in analysis time, combined with the ability to deliver a closed "sample in-answer out" multi-process system within a single device, preventing sample loss and contamination.[1] Microfluidics presents an eventual economical option, with the ability to rapidly fabricate plastic microdevices using CO_2 laser ablation. In addition, using infrared-mediated heating and fan-assisted cooling, PCR is completed in half the conventional time, with instrumentation costing a fraction of that for a block thermocycler.[2] Progress toward a fully-integrated device has become simpler with the recent development of direct blood amplification, enabling the removal of the DNA extraction domain within the microdevice.

Here, a master mix capable of this chemistry, combing a commercially-available neutral proteinase and *Pwo* DNA polymerase, enabling customization of the components is presented. Initially, this was demonstrated in a conventional 25µL tube PCR reaction, amplifying the TPOX allele using 1µL of whole blood as the template. The PCR product was separated on a microchip electrophoresis platform, demonstrating successful amplification of the alleles within the TPOX locus. Since *Pwo* is a high-fidelity polymerase, no non-specific amplification was observed, which was seen as problematic with an alternative direct blood PCR kit. In addition, the successful amplification of the TPOX locus was achieved from 1µL blood stains on filter paper, cotton, cotton-polyester, and demin substrates. To confirm this amplification was human-specific, blood samples from a rhesus monkey, a rabbit, a mouse, and a dog were processed in the same manner, resulting in no peaks, confirming the specificity of the assay for human samples.

Translating this direct tube-based PCR method to a microdevice has the potential to significantly decrease the time required for the reaction, and if this could be linked to a label-free read-out, integration would allow for immediate results without further sample handling. Recently, a visual, label-free end-point detection that utilizes hybridization of biotinylated oligonucleotide probes bound to 1µm streptavidin-coated paramagnetic particles to the complementary (target) sequence was reported.[3] A rotating magnetic field agitates the particles, exposing the bead-bound oligonucleotide for hybridization to the target sequence to form an aggregate; visual detection of the aggregates confirms the presence of the target DNA. This is visually distinguishable from sample without target DNA where the particles remain dispersed. Cellular debris associated with the enzyme-based DNA liberation step did not result in any false aggregation, confirming that the particles only aggregate when adequate target copies are available as a result of successful amplification. This circumvents the requirement for fluorescent tags, an amplicon separation step, and skilled labor, while providing a highly sensitive, accurate method for specific DNA sequence detection in less than two minutes.

In summary, the rapid detection of a fragment of the TPOX gene directly from whole blood on a single device is facilitated by a microfluidic network that links the PCR and an aggregation domain, and requires no external apparatus to control fluid flow. This work presents a total micro analysis system, capable of identifying the presence of the TPOX allele from human blood using a plastic microdevice, integrating single chamber, infrared-mediated, direct PCR and visualized aggregation, in 60 minutes.

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Direct PCR, Microdevice, Human Identification

A79 Development of a Direct Amplification Method for Exemplar and Pseudo-Exemplar Reference Samples Using Identifiler® Plus

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The goal of this presentation is to explore methods of direct PCR amplification for processing exemplar and pseudo-exemplar reference samples.

This presentation will impact the forensic science community by evaluating methods of direct PCR amplification that eliminate the need for purification and quantitation, greatly reducing the time and cost for analysis, offering DNA analysts a more effective means of processing reference samples.

Pilot studies comparing the AmpFℓSTR® Identifiler® Direct PCR Amplification Kit and AmpFℓSTR® Identifiler® Plus PCR Amplification Kit were conducted to determine which amplification system best fits the needs of the Office of Chief Medical Examiner of New York City. The goal of these experiments was to establish a standard protocol that will account for variability of DNA amounts among reference samples.

Identifiler® Direct is a PCR amplification kit optimized for amplifying blood samples spotted onto FTA® cards that have not been extracted or quantified. Use of non-FTA® collection methods, according to Applied Biosystems (ABI), requires pretreatment with Prep-and-Go Buffer™. To potentially bypass this step, various sampling techniques and thermal cycling parameters were tested. The ABI GeneAmp® PCR System 9700 thermal cycler and the ABI 3130xl Genetic Analyzer were used for amplification and separation, respectively, and all data was analyzed with GeneMapper® ID v3.2.1. Initial results showed that partial profiles were obtained for buccal samples and non-FTA blood cards. These profiles displayed many N-bands. Due to the additional time required to process samples using the Prep-and-Go Buffer™, other less costly amplification kits were then considered.

Another method of direct PCR amplification utilizes Identifiler® Plus, which is optimized to overcome inhibition. The chemistry of the kit enables unpurified extracts to be directly amplified. Buccal swabs and blood spotted onto Whatman® non-FTA® paper were incubated in 0.2% Tween® 20, 0.1mg/mL Proteinase K, and 2.4% Trehalose in TE^{-4} for 30 minutes at 56°C followed by five minutes at 99°C. An aliquot of neat extract was directly amplified using a half reaction of Identifiler® Plus (5.0µL Reaction Mix, 2.5µL Primer Set). Experiments with various cutting sizes, extraction volumes, aliquots for amplification, and thermal cycling parameters were conducted using samples containing a wide range of DNA. In order to generate results for all samples, the 29-cycle protocol was implemented. In addition, the final elongation time was increased to 60 minutes to minimize N-bands observed at Amelogenin only. Full profiles were obtained for all samples tested with only 30% of the samples needing an additional injection on the 3130xl Genetic Analyzer at lower parameters due to oversized peaks.

Reproducibility, sensitivity, and stability were also evaluated. A large number of different donors were tested, each in duplicate on different days and using different instruments. To decrease the amount of DNA collected, donors were instructed to abbreviate the amount of time they swabbed their mouths. More controlled sensitivity studies with known amounts of DNA were also performed. In order to compromise buccal specimens, swabs from previously tested donors were stored under accelerated aging conditions. Although for most samples, protocol adjustments were unnecessary, in a few cases with very low yields of DNA, the amplification aliquot volume was increased.

Methods were also optimized for pseudo-exemplar samples such as bottles, cans, cups, straws, cigarette butts, and chewing gum. One uniform protocol that encompassed all of these sample types was developed with the exception of different-sized cuttings for each substrate. Preliminary results showed that over 98% of all samples yielded full profiles, with 15% requiring a second injection at either a higher or lower parameter on the 3130xl Genetic Analyzer.

In brief, implementing a short extraction step followed by direct amplification with Identifiler® Plus proved to be a cost-effective method to profile true and pseudo exemplar samples in a single day or less. For the overwhelming majority of samples, full profiles were generated using standard parameters. For a select number of samples, an additional injection on the 3130xl Genetic Analyzer with more or less sensitive parameters and/or increased template volume for amplification was required. In both cases, the initial STR results could be evaluated to accurately predict the additional step(s) needed to preserve both time and cost.

Direct Amplification, Exemplar, DNA Analysis

A80 An Evaluation of Direct PCR Amplification

Reena Roy, PhD, Penn State Univ, Forensic Science Program, 325 Whitmore Lab, University Park, PA 16802; and Daniel E. Hall, BS, Penn State Univ, Forensic Science Program, 107 Whitmore Laboratory, University Park, PA 16802*

After attending this presentation, attendees will understand the concept of direct amplification from nine different substrates. Attendees will also appreciate the method that allows the scientist to generate autosomal and Y-STR profiles from substrates without the cumbersome steps of extraction and quantitation.

This presentation will impact the forensic science community by informing the forensic DNA analyst of a faster, less expensive approach to obtaining DNA profiles from bodily fluids using eight commercially available amplification kits.

The objective of this research was to generate complete autosomal STR profiles from body fluids using direct amplification and various commercially available STR amplification kits. Attempts were also made to detect the Y-profile from male body fluids using 1.2mm punches and the AmpFℓSTR® Yfiler™ kit following direct amplification.

STR analysis of blood and buccal samples is often used in the fields of forensic biology and genetics for casework, paternity testing, and convicted-felon DNA profiling. A primary advantage of direct amplification without purification of DNA is the high throughput of databank samples. Direct amplification of DNA stored on various types of substrates reduces the time required to obtain a DNA profile, thus reducing cost and increasing efficiency.

FTA® cards are suitable for criminal offender DNA database samples and casework reference samples. The preservative in these cards contains proprietary chemicals to protect DNA molecules from nuclease degradation, and to protect the host matrix from bacterial growth. DNA from biological samples deposited on FTA® paper and similar commercial storage devices has been found to be stable for a period of several years when stored at ambient temperature. A primary advantage of FTA® paper and similar substrates is their ability to provide consistent results without quantification, and the procedure can be automated.

Direct amplification kits contain improved PCR buffer cycling protocols that can overcome inhibitors during PCR amplification steps. Most collection media for storing dried body fluid samples contain chemicals that are capable of lysing cells (which may contain PCR inhibitors) to preserve DNA within a sample. Using devices without lysing chemicals, such as the Bode DNA Collector, requires an additional lysis step, or else amplification quality may be poor and allelic dropout may occur. Some of the amplification kits listed above have been optimized with enhanced reagents for direct amplification. However, it may not be cost effective for crime laboratories to use these direct amplification kits. Kits such as the PowerPlex® 16 System and the AmpFℓSTR® Yfiler™ Amplification Kit require the addition of polymerase in the reaction.

The following nine collection media were used for this study: proPRIME*i* Indicating Micro, 705 Micro, Blood Direct #1 and #2, Collection Card, CEP swab from FITZCO, EasiCollect from Whatman, FTA Indicating Micro, and Bode DNA Collector. Blood from two deceased individuals and saliva from three living donors were used in this study. The three single-source saliva samples and two single-source blood samples were deposited on each of the 45 collection devices.

A 1.2mm punch of each of the 45 substrates containing one body fluid was amplified with PowerPlex® 18D, PowerPlex® 16 HS, PowerPlex® 16, and PowerPlex® 21 Systems from Promega Corporation, and AmpFℓSTR® Identifiler® Direct, Identifiler® Plus, and Identifiler® PCR Amplification Kits from Applied Biosystems following each manufacturer's recommended conditions. Similarly, 1.2mm punches of the substrates containing male body fluids were amplified with AmpFℓSTR® Yfiler™ PCR Amplification Kit.

Results from the eight kits mentioned above were compared. Both blood and saliva samples appeared to yield complete DNA profiles. Two different reaction volumes were attempted with substrates that yielded

complete profiles from single source samples, the first using the manufacturer's recommended volume, and the second using half of the reaction volume suggested in the protocol. For some of the substrates, thermal cycling conditions were modified as necessary to generate complete DNA profiles.

Another goal of this research was to demonstrate that direct PCR amplification can be applied to commercially available kits not intended for direct amplification. The results indicate that it is possible to do so.

Direct Amplification, STR, Y-STR

A81 Protocols for Rapid Amplification of STR Typing Kits: The Use of "Non-Standard" Thermal Cyclers

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After attending this presentation, attendees will understand the principles of rapid PCR amplification of STR loci, characteristics of rapid PCR thermal cyclers, and applications of rapid DNA typing.

This presentation will impact the forensic science community by detailing protocols used for rapid PCR amplification and how these methods can be implemented into forensic laboratories and commercial integrated forensic DNA typing devices. The potential of reducing the required PCR amplification time may also benefit laboratories typing single-source reference samples.

There is a growing interest in developing methods capable of processing a single-source reference sample (e.g., a buccal swab) to an STR profile in under two hours. The ability to develop a protocol for the rapid typing of forensic STR markers is of specific interest to the forensic DNA and biometric communities. This would allow for a faster sample-in-answer-out turnaround time as well as the potential for higher throughput capabilities. One critical component of a rapid workflow involves the reduction in time required for multiplex PCR amplification of the core STR loci.

Initial tests of various commercial thermal cyclers with two- and three-step PCR cycling protocols were carried out and compared for genotype accuracy and DNA template sensitivity with a set of previously extracted single-source DNA templates. Work with various "non-standard" thermal cyclers in combination with faster processing DNA polymerases has resulted in decreasing the PCR amplification time to less than 20 minutes for a 16 locus commercial DNA typing kit.

Approximately one nanogram of DNA template was amplified in a total volume of 10 microliters. The PCR primers used for the amplification came from a commonly used commercial STR typing kit, and were used without any further modifications. Both the two- and three-step PCR protocols employed a hot start at 95 degrees for one minute for the activation of the DNA polymerase. The cycling parameters for the 2-step protocol consisted of 28 cycles of 95°C for five seconds followed by 61°C for 15 seconds. The cycling parameters for the 3-step protocol consisted of 28 cycles of 95°C for 5 seconds, 58°C for 10 seconds, followed by 72°C for 10 seconds. Both protocols employed a 72°C incubation step for one minute post cycling to promote complete PCR amplicon andenylation. The required cycling time for the two- and three-step PCR thermal cycling protocols ranged from 15-36 minutes depending on the specific thermal cycling being tested.

The rapidly generated PCR products were then diluted into a formamide solution and prepared for separation and detection by capillary electrophoresis. At least 15 unique samples were typed for the evaluation of each rapid PCR protocol on each of the thermal cyclers. The analysis of capillary electrophoresis data of the PCR products indicated good peak balance for heterozygous loci (median values were greater than 0.8), strong signal intensity (on average over 1,000 relative fluorescence units on a commonly used peltier block thermal cycler) and minor adenylation and PCR artifacts. Stutter artifacts were not significantly different when comparing the two- and three-step thermal cycling protocols. Genotyping results were concordant with PCR amplification conditions utilizing standard thermal cycling procedures (which require at least three hours).

The PCR conditions and cycling parameters developed were robust enough to routinely amplify 250pg of template DNA. These conditions can potentially be applied in a laboratory setting for faster generation of STR profiles while maintaining the robustness and reliability required by the forensic typing community.

PCR, Rapid DNA, Forensic DNA Typing

A82 Evaluation of Direct PCR for Forensic DNA Profiling and the Development of a Direct PCR Multiplex

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After attending this presentation, attendees will understand the basic principles of direct PCR and how it can be used in the analysis of forensic DNA profiling. Other items for discussion include the use of direct PCR with various types of samples retrieved from commonly encountered porous and non-porous substrates, the effect these substrates have on the recovery of DNA, and, finally, the development of a novel multiplex that has been validated to be used with direct PCR.

This presentation will impact the forensic science community by providing insight into the benefits of using direct PCR compared to conventional DNA profiling protocols to analyze various biological samples for DNA profiling. This research also focuses on the development and validation of the direct PCR multiplex for the simultaneous amplification of autosomal and Y- STRs, and two internal PCR controls for the identification of sample quality.

The goal of this research was to evaluate the use of direct PCR with different types of biological samples and to develop a direct PCR multiplex. Direct PCR is a technique in which DNA samples are subjected to PCR without having to first undergo extraction and quantification. With different extraction techniques, there is an associated loss of DNA which is caused by extraction inefficiencies. The multiple tube changes during extraction also introduce the opportunity for contamination and handling errors. With direct PCR, better DNA profiles could be obtained faster and cheaper as there is no loss of DNA associated with extraction steps and it does not use expensive commercial extraction and quantification kits. In this study, genomic DNA preparations and buccal cell counts of various concentrations were deposited on commonly encountered substrates, recovered, and amplified using direct PCR before subjecting them to capillary electrophoresis. The electropherograms obtained were compared to those obtained using the standard DNA profiling protocol which involves extraction prior to amplification and fragment analysis. Direct PCR was found to be more successful than the conventional DNA profiling protocol and was further tested with fingerprints, touch DNA on fabric, and blood and semen stained fabrics. All these tests were successful with direct PCR, indicating that this technique has the potential to be incorporated into routine forensic DNA testing. Supplementary tests were carried out to compare the efficiency of the swabbing technique utilized throughout this study and the effect that different substrates had on DNA recovery. Four non-porous substrates—glass, stainless steel, plastic, and ceramic—and four types of dyed fabrics—white cotton, light blue denim, nylon, and brown cotton—were used and the resulting DNA profiles were evaluated. The results obtained from this experiment indicated that the substrate on which DNA is deposited affects the amount of DNA retrieved, and, subsequently, the generation of DNA profiles. Several commercial multiplexes were used to amplify these samples using direct PCR and it was found that some of these multiplexes were more suitable than others for amplifying using direct

PCR and several pitfalls were identified. With this in mind, a novel multiplex consisting of five autosomal and two Y-STRs, which also provides the inhibitor status of the sample, was developed and validated with direct PCR samples. This multiplex also addresses the issues of sensitivity and robustness that were encountered with the commercial multiplexes tested. This direct PCR kit successfully amplified various mock crime scene samples without prior extraction, while being able to amplify as low as 25pg of pristine DNA. Allelic ladder, panels and bins were created to be used with this multiplex to aid in sample designation when subjected to capillary electrophoresis.

Direct PCR, DNA Profiling, Multiplex Development

A83 Field Forward Rapid DNA Analysis Outside the Laboratory: A Ruggedized System for Fully Automated Generation of STR Profiles

Eugene Tan, PhD, NetBio, 830 Winter St, Waltham, MA 02451*

After attending this presentation, attendees will learn of recent advances in rapid DNA analysis systems that enable forensic sample analysis to be performed rapidly in field-forward settings, mechanisms for long-term reagent storage at room temperature to support field-forward operation, and instrumentation features that enable transportation to and operation of rapid DNA analysis instruments in the field.

This presentation will impact the forensic science community by introducing a fully integrated, samples-in to answers-out short tandem repeat (STR) analysis system that offers the potential for use in a wide range of out-of-laboratory settings, including police stations, borders and ports, military checkpoints, and the battlefield.

Applications for the generation of STR profiles in the field include forensic investigations, determination of battlefield friend or foe, verification of kinship in immigration cases, and victim identification at mass casualty sites. To fully impact these applications, however, Rapid DNA Analysis systems must be ruggedized to allow transport and field-forward operation. Critically, all system reagents must be stable at room temperature for extended periods as many of the projected sites of operation are not amenable to refrigerated storage.

The system to be presented is immediately operational with full functionality after transport and capable of generating STR profiles from buccal samples in approximately 83 minutes without the need for a technical operator. To enable room temperature storage and stability, all reagents are stored within a single-use BioChipSet. Certain reagents, including the PCR reaction mix and Internal Lane Standard are lyophilized. The remainder, including electrophoresis and guanidinium-based purification reagents, are stored in liquid form in sealed aliquots within the BioChipSet. The liquid reagents are automatically released and the lyophilized reagents are automatically resuspended during sample processing. For example, the lyophilized PCR reaction mix is resuspended by an aliquot of the purified DNA solution generated during processing. Approaches to reagent storage and automated release will be discussed.

The Rapid DNA Analysis system was designed to be readily transported to and operated in the field. The instrument can be uncrated and set up in less than 15 minutes, is ready for use without any recalibration or adjustments, and can be operated with AC voltage between 90V to 260V (50 or 60 Hz) using utility or generator power. Several design features have been incorporated to provide ruggedization for field-forward operation including: (1) shock isolation and vibration dampening features in the mechanical chassis to protect the highly sensitive optical subsystem; (2) temperature control features to maintain electrophoresis run temperature to within one degree even with wide swings in ambient temperature; (3) lane-finding software to automatically align the optical detection system for each run; and, (4) partitioning of subsystems to protect against shock and vibration. The completed system was tested to MIL-STD 810F for transport. These tests included:

- Transit Shock – The instrument was crated, raised 12" above a solid concrete floor and dropped as prescribed in the test procedure.
- Bench Shock – The instrument was placed on a laboratory benchtop and raised on one side with by 4" and released, as prescribed in the test procedure.
- Vibration Testing – The crated instrument was placed on a vibration table and exposed to the vibrational frequencies for truck transportation as prescribed in the test procedure.

Data for the MIL-STD tests, automated subsystem tests, and functional tests will be presented to demonstrate that the system meets or exceeds all requirements for STR analysis in field-forward operation.

Rapid DNA, Ruggedization, MIL STD 810F

A84 Rapid DNA Analysis: Fully Integrated, Fully Automated Generation of STR Profiles From Buccal Swabs

Eugene Tan, PhD, James W. Schumm, PhD, and Richard Selden, MD, PhD, NetBio, 830 Winter St, Waltham, MA 02451*

After attending this presentation, attendees will have learned how fully-automated STR profile generation from buccal swabs can be performed without a technical operator in the laboratory, police station, or field-forward settings.

This presentation will impact the forensic science community by offering a fully integrated, samples-in to answers-out Short Tandem Repeat (STR) analysis system that offers the potential for use in a wide range of out-of-laboratory settings, including police stations, borders and ports, military checkpoints, and the battlefield.

This presentation describes development of a Rapid DNA Analysis system consisting of a modular platform that allows customization to perform a wide range of nucleic acid analyses. Independent microfluidic modules developed include those for DNA purification, DNA quantitation, highly multiplexed amplification, DNA sequencing, electrophoretic separation and detection, and related control, analytical, signal processing, and expert system profile determination software. The system consists of a fully automated instrument and a single accompanying BioChipSet cassette that can be used by non-technical personnel in laboratory, office, or field-based settings while dramatically reducing the time required to perform STR analyses. This presentation summarizes the successful application of the methodology in generating CODIS-quality DNA profiles in 83 minutes from buccal swabs without human intervention.

The Field-Deployable Accelerated Nuclear DNA Equipment ("ANDE") is operated by inserting five buccal swab samples into a BioChipSet, placing the BioChipSet into the instrument, and pressing the start button. The instrument provides all the subsystems required for the completion of STR analyses, including the power, thermal cycling, pneumatic, optical, ruggedization, process control, and computer subsystems. The instrument interfaces to the BioChipSet using a number of features, including a pneumatic manifold to allow fluids to be driven, thermal features to maintain appropriate temperatures during PCR and electrophoresis, optical paths to allow excitation and detection of separated STR fragments, and electrical connections to support electrophoresis. Attendees will become familiar with several critical features of the instrument and BioChipSet including:

- The BioChipSet contains all reagents on-board, factory pre-loaded. The user does not load or otherwise handle reagents. Several reagents are lyophilized (e.g., amplification reaction mix) and others are in liquid form (e.g., purification reagents). All reagents are stable at room temperature.
- The BioChipSet is closed: each buccal sample is processed through its own sealed processing path and samples and reagents do not have any contact with the instrument itself.
- To limit handling requirements, the BioChipSet is a single disposable plastic component. The operator has nothing to connect. No opening, filling, or other handling of the

disposable piece is required, minimizing the possibility of cross-contamination.

- Buccal swabs lock into separate purification positions so that once loaded they cannot be moved to another location. An RFID chip in the swab cap is detected by the instrument lid to identify the swab location within the BioChipSet.
- The instrument is ruggedized to MIL STD 810F for shock and vibration. This allows it to be moved within the forensic laboratory, transported for use outside of the laboratory, or used in a police station or field-forward setting. Instrument autocalibration readies the instrument for use within 15 minutes of setting it up.
- The instrument contains an on-board computer and touch screen monitor for interfacing with the operator, and the instrument's wireless, USB, and Ethernet connectivity options can be configured to user requirements. Also based on user requirements, the system includes an expert system for conversion of electrophoretic traces to CODIS/NDIS-compatible profiles and .cmf output, GPS-tagging of data products with time and location data, and an internal database to store instrument-generated profiles.

Description of the instrument design, the processes conducted in automated processing, and data characterizing the output generated from the fully integrated multiplex STR instrument-biochipset-expert system format will be presented.

Rapid DNA, Expert System, ANDE

A85 A Fast STR Genotyping Process for Time-Sensitive Situations

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After attending this presentation, attendees will learn about different approaches aimed at reducing the time to generate STR profiling results, including a short lysis step, extraction on the Promega Maxwell 16 System and rapid PCR protocols, and about their integration into a single accelerated analytical process.

This presentation will impact the forensic science community by providing options and feasible alternatives for laboratories interested in gaining analytical efficiencies or to expedite sample processing, offering strategies compatible with standard forensic laboratory equipment.

Significant efforts are being devoted to the development of methods enabling rapid generation of short tandem repeat (STR) profiles in order to reduce turnaround times for the delivery of human identification results from biological evidence. Moreover, in some circumstances, the need for rapid human identification might be critical for police investigations. Different approaches were investigated to achieve flexibility for processing biological evidence. Combining these accelerated protocols into a single analytical process enables generation of STR profiles for human identification within five hours.

Reduced incubation times at 56°C for the lysis of biological samples were evaluated (30 min to 4 hours) and the results were compared to the usual overnight incubation. A quick DNA extraction method using the Promega Maxwell 16 System (27 min/16 samples) was also explored and the results were compared to the RCMP automated DNA IQ extraction protocol on TECAN robotic workstations. A variety of single-source and two-person mixture samples were subjected to different lysis conditions and extraction methods. With the exception of old blood on FTA cards, a 30-min incubation was sufficient to obtain similar or higher DNA yields compared to the longer incubation times. DNA yields were enhanced for some challenging fabrics, likely due to a reduced quantity of dye/chemicals leaching out of the fabric and interfering with the extraction by DNA IQ. While comparable STR profile quality was usually obtained, improvement in inter-loci balance was noted for the 30-min lysis step for saliva and buccal samples. Similarly, DNA yields and profile quality were shown to be equivalent using the quick DNA extraction method on the Promega Maxwell 16 when compared to the current RCMP DNA IQ method.

By modifying the cycling conditions and combining the use of a DNA polymerase optimized for high-speed PCR (SpeedSTAR HS) and of a more efficient thermal cycler instrument (Bio-RAD C1000), it was possible to reduce the amplification process time to 26 minutes. No modification to the commercial AmpFℓSTR® Profiler Plus or Identifiler primer mix was required. Mock and casework single-source and two-person mixture samples were used. Compared to standard amplification protocols, the fast amplification procedure demonstrated similar sensitivity, peak height ratios, and overall profile balance. Minor alleles in mixtures were reliably typed. An increase in the n-4 stutter ratio (2.2% on average for all loci) for profiles amplified with the fast protocol was noted compared to the standard protocols. Complete concordance was obtained with profiles previously generated with a standard amplification protocol for mock case samples and for casework samples.

Together, these protocol modifications provide interesting options to current laboratory processes to expedite the generation of STR results, especially in circumstances requiring quick actions. Other strategies to further reduce turn-around time included the evaluation of the Qiagen Investigator HYres quantification kit and profile generation on an Applied Biosystems 3500/3500xl Genetic Analyzer. The cycling procedure of the Investigator HYres cuts off approximately 30 minutes in the quantification step compared to the Applied Biosystems Quantifiler Duo. Comparable human and male DNA yield values were obtained for both systems from forensically relevant DNA samples. Excellent STR profile quality was demonstrated from biological samples submitted to the entire accelerated analytical process in less than five hours.

DNA, STR, Accelerated Process

A86 How Distant Relatives Influence the Efficiency and Error Rate of Familial Searching

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After attending this presentation, attendees will gain a sophisticated understanding of familial searching arising from realistic population genetics and the presence of distant relatives in DNA databases. Since the utility of familial searching is predicated on relatives being present in these databases, it is important to consider how these relationships affect the practical application of familial searching techniques. Specifically, attendees will see how while familial searching effectively identifies first-degree relatives, distant relatives are also very commonly misidentified as first-degree relatives, calling into question the effectiveness of large database familial searching.

This presentation will impact the forensic science community by bringing to light important caveats affecting familial searching, which may lead to increased false identification rates. Depending on the demographics of DNA databases, these false identification rates may be unacceptably high, potentially influencing decisions about the use and implementation of familial searching methods. This is particularly relevant considering the continuing rapid rise in use of familial searching.

Familial searching is now being implemented in several states and is under consideration for national application as a tool to aid cold case investigations. The consequences of adopting the familial searching criteria used by the state of California, as described by Myers *et al*., (2011) are considered.[1] A simulation study, in which randomly generated profiles of related and unrelated individuals, comprising of a 13-locus CODIS genotype and YFiler® Y-chromosome haplotype, were considered for first-degree relationships as carried out through the Myers protocol for relative identification, was conducted. The Myers protocol powerfully identifies first-degree relatives who share a Y-

chromosome, with an 80-99% probability, depending on the population background of the individuals in question. For unrelated individuals, there is a low probability of false identification as first-degree relatives. The Myers protocol showed low probabilities of falsely identifying totally unrelated individuals as first-degree relatives, supporting the method to distinguish first-degree relatives from unrelated individuals. However, for more distant Y-haplotype sharing relatives (half-siblings, first cousins, half-first cousins, or second cousins), there is a substantial probability of incorrect identification as first-degree relatives. For example, there is a 3-18% chance of identifying a first cousin as a full sibling, with the probability depending on the population genetic background of the individuals in question. The California familial search policy is likely to identify a first-degree relative if their profile is in the database and it poses little risk of falsely identifying an unrelated individual in a database as a first-degree relative. However, importantly, there is a substantial risk that a somewhat more distant Y-chromosome sharing relative whose profile is in the database will be wrongly identified as a first-degree relative, with the consequence that their immediate family will become the target for further investigation. In this outcome, the familial search is ineffective in identifying the true relative and may convincingly lead investigators to futilely consider distant relatives who may not even be aware of their relationships to the true relative in question. Importantly, this risk of unprovoked investigation falls disproportionately on those groups that are over-represented in state and federal databases, particularly African Americans and Latinos.

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Familial Searching, Error Rates, Population Genetics

A87 NIST STRBase Resources to Aid Work With New STR Kits and Loci

John M. Butler, PhD, NIST, 100 Bureau Dr, MS 8312, Gaithersburg, MD 20899*

After attending this presentation, attendees will learn about the creation of resources on the STRBase website to aid the understanding of new DNA tests, developed after new short tandem repeat (STR) loci were required for European and U.S. forensic DNA databases.

This presentation will impact the forensic science community by describing online resources under development at the National Institute of Standards and Technology (NIST) that supports work with new DNA tests.

With new short tandem repeat (STR) loci being required for European and U.S. forensic DNA databases, a number of new DNA tests have been developed in the past few years. Attendees will learn what resources exist on the STRBase website to aid their understanding of these new DNA tests.

For the past 15 years, NIST has maintained the Short Tandem Repeat DNA Internet DataBase (STRBase), which is located at http://www.cstl.nist.gov/biotech/strbase. The purpose of STRBase has been and continues to be an attempt to bring together the abundant literature and information in the forensic genetics field in a cohesive fashion in order to make current and future work easier. New materials are regularly added to expand the information contained on the STRBase website.

Information on the STRBase website is contained in hypertext markup language (HTML) files that were created primarily using a editor and website administration tool. Over 1,900 files now exist containing more than 10,000 printed pages of information that are connected with over 5,000 hyperlinks. An additional 2,300 hyperlinks connect various information on STRBase to other internet websites, including over 350 direct links to various organizations, journals, academic and forensic institutes, commercial sites, genetic genealogy labs, parentage testing labs, and legal sites dealing with forensic DNA (see http://www.cstl.nist.gov/biotech/strbase/weblink.htm). Thus, STRBase is not a true searchable "database" but rather a collection of information with interconnected files. Since July 2006, resources that are added to STRBase are now tracked on a "recent updates" page: http://www.cstl.nist.gov/biotech/strbase/updates.htm.

STR fact sheets are the centerpiece of STRBase. These fact sheets list information regarding genomic location, GenBank accession, repeat structure, reported PCR primer sets, observed allele sizes and sequence structure, commercially available allelic ladders, common multiplexes, and mutation rates. All 24 core or common STR loci used in current commercial STR kits are available. Multiplex assay and kits are visually summarized by locus-specific allele size ranges and dye colors with each locus name hyperlinked to the appropriate STR fact sheet. Labs worldwide continue to contribute to knowledge regarding rare alleles such that we now have catalogued over 600 variant alleles and 300 tri-allelic patterns.

Numerous presentation slides, NIST publications and presentations, software programs, and other useful information are available for download and use by the forensic genetics community. Materials from more than 40 recent workshops consisting of thousands of slides covering capillary electrophoresis, low-copy number DNA testing, mixture interpretation, qPCR DNA quantitation, Y-chromosome and mtDNA analysis, and validation are available for use at http://www.cstl.nist.gov/biotech/strbase/training.htm.

NIST's Applied Genetics Group has also updated NIST Standard Reference Material (SRM) 2391c with certified and informational values for commonly used autosomal and Y-STR loci. The information available from NIST's latest SRM will be discussed.

STRBase has been well received and widely used by the forensic DNA community and additional resources for the website continue to be created. In 2005, NIST adopted STRBase as an official Standard Reference Database (SRD) giving further credence to the value of the information contained in the website.

Forensic DNA, STRBase, Internet Resources

A88 Results of DNA Processing of Unselected Sexual Assault Evidence

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The goal of this presentation is to offer an overview of the results of sexual assault kit processing and compare the final disposition of cases with the DNA results obtained.

This presentation will impact the forensic science community by offering a comparison to studies of testing of backlogged sexual assault kits stored for long periods by law enforcement agencies at a variety of locations in the country.

Sexual assault kit backlogs are at the forefront of controversy in the forensic science community. A number of jurisdictions across the country have reported backlogs of thousands of untested sexual assault kits in law enforcement property rooms or laboratory evidence storage. There are differing opinions as to the usefulness of forensic testing in these backlogged cases. Community groups, victim's rights advocates, and some victims have called the backlogs justice delayed or denied. Studies by others are underway to test the backlogged evidence and to determine if timely analyses would have been helpful. A retrospective study of the testing of all sexual assault evidence without delay collected in the jurisdictions served by the Forensic Genetics Laboratory of the Harris County Institute of Forensic Sciences (HCIFS) is presented.

The HCIFS serves approximately 2,000,000 of the 4,000,000 people in Harris County outside the city of Houston, providing forensic DNA testing services to more than 60 law enforcement agencies. HCIFS policy requires all collected sexual assault kits to be submitted directly to the laboratory without being sent first to the property room of the submitting agency. HCIFS tests all evidence within approximately 60 days and has no backlog of untested kits. The purpose of this study is to identify the outcomes of a set of completed and adjudicated sexual

assault cases. The study is unique in that it includes *all* collected sexual assault cases, not just those for which DNA testing was not originally requested.

Cases received during 2010 were assessed for this project. For each of these cases, information was gathered from the complainant's narrative from the kit's medical report, law enforcement offense records, DNA reports, CODIS hit information, and court records. Data included whether a sexual assault kit was collected and the amount of time that elapsed between the assault and the evidence collection. Complainant and suspect information included their ages and relationship, if any. The assaults were categorized by a large number of variables including whether there was evidence of drug-facilitated sexual assault, whether the suspect ejaculated, and whether the victim knew the attacker. DNA results documented whether a suspect known was submitted for comparison, whether semen and/or male DNA was detected, and whether the suspect known matched a profile in the evidence. Additionally, the final disposition of the case and the number of CODIS offender hits and forensic hits were also tracked.

Preliminary data indicates that foreign DNA was detected in 60% of cases. A profile was entered into CODIS in 83% of these cases. A CODIS offender hit or forensic hit was returned in 24% of the cases, and 45% of the offender hits in these cases were to offenders not identified during the investigation. The preliminary data show that charges were filed in 35% of the cases. Conference attendees will be provided comprehensive information from the data set. The value of DNA testing of all sexual assault kits collected versus testing of only selected kits will be discussed.

Sexual Assualt Kit, DNA, Backlog

A89 Processing One Million DNA Samples: Lessons Learned From a Decade of Offender Databasing

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After attending this presentation, attendees will learn: (1) a variety of tools and methods that can improve the efficiency and quality of offender databasing processing; (2) the importance and impact of selecting the most appropriate procedures and technical specifications; and, (3) how to recognize factors that significantly affect turn-around time, cost, efficiency, and overall productivity within their own forensic DNA laboratories.

This presentation will impact the forensic science community by discussing methods that DNA databasing laboratories can use to increase productivity, improve quality, and decrease costs in order to effectively manage and reduce offender DNA sample backlogs.

As a result of expanding offender collection legislation and demands to reduce DNA backlogs across the country, laboratories are continually confronted with meeting the challenges of an increased workload while following strict budgetary constraints. Additionally, with the increase in arrestee legislation, there is a higher demand to process DNA reference samples in much shorter turnaround times, which can often lead to inefficiencies within the laboratory. Through the processing of more than one million DNA database samples for the U.S. national database (CODIS), and more than 10 years of experience in high throughput DNA database sample processing and analysis, this study has identified a variety of methods described in this presentation that other laboratories can use to eliminate time-consuming and costly inefficiencies to increase throughput and reduce DNA backlogs.

This presentation will address ways to optimize the collection and testing process, and improve the first pass success rates of databasing samples. Maximizing the first pass success rate allows the laboratory to minimize re-testing, re-amplifications, re-injections, and ultimately simplifies data review. In order to achieve full optimization, a variety of processing, analytical, and quality factors were identified and evaluated based on overall impact and ease of implementation within the laboratory. Substrate options were carefully examined and evaluated, and a variety of extraction techniques were compared to determine which methods yielded the highest quality DNA while minimizing cost and processing times. Additionally, through experience, testing, and cost analysis reports, it was determined that maintaining a proper balance of automated and manual procedures is more productive and efficient than relying on just one method.

The selection of appropriate technical specifications is also highly critical. Specifications such as imbalance ratios, minimum relative fluorescent unit (RFU) values, and ceiling thresholds should be carefully considered. These factors can be key drivers of first pass success rates and dramatically influence reprocessing rates and cost. In addition, the importance of implementing a Laboratory Information Management System (LIMS) program that has been specifically designed for high-throughput processing of databasing samples is explained. The use of an LIMS program allows a laboratory to effectively track samples starting from accessioning through analysis and reporting, and should include extensive quality control checks specific to high-throughput testing. Additionally, the number of samples processed together within a batch has a large impact on cost and productivity. Factors such as collection rates, turnaround times, and staffing should be carefully considered when determining the appropriate batch size both for in-house processing and for outsourcing.

Knowing the limits of each step can also aid in the development of an effective database analyst training program. It has been documented that new analysts can be effectively trained within six weeks and be fully independent once the FBI QAS six-month training requirement is fulfilled. Finally, analyst productivity tracking has shown that analysts will be approximately 40% more efficient in their second or third year than analysts in their first year. This determination facilitates project planning, personal development goals, and the ability to meet expectations in even shorter turnaround times.

Database, Efficiency, Backlog

A90 The Impact and Benefit of Expanding the U.S. Core Autosomal STR Markers

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After attending this presentation, attendees will: (1) understand the benefit and potential impact of expanding the U.S. core autosomal STR markers for DNA database searches; and, (2) get an overview of the next-generation multiplex kits that include these additional markers.

This presentation will impact the forensic science community by demonstrating how DNA database searches can be benefitted by additional STR loci.

The original core set of 13 Combined DNA Index System (CODIS) autosomal short tandem repeat (STR) loci were selected in November 1997 and are required by the Federal Bureau of Investigation (FBI) for upload of DNA profiles to the national DNA database.[1,2] As the number of profiles stored in the National DNA Index System (NDIS) continues to increase each year (>10 million total profiles), the likelihood of adventitious matches becomes greater. Expanding beyond the 13 core loci is critical to reduce the potential of these types of matches occurring within the database, to increase international compatibility for data sharing, and to increase discrimination power in missing persons cases.[3]

In November 2009, the European Union adopted five new autosomal STR loci as part of their expanded European Standard Set (ESS), including D12S391, D1S1656, D2S441, D10S1248, and D22S1045. These new ESS STR loci were selected based on discussion over the past few years within the European Network of Forensic Science Institutes (ENFSI).[4,5] Unfortunately, only eight of the current 13 U.S. core loci overlap with data being gathered in the United Kingdom and most other European nations. All five of these new loci are being considered for the expansion of the U.S. core set to provide greater capabilities for international comparisons when necessary. Also,

D2S1338 and D19S443 are recommended as two new additions to the original 13 core loci because they are commonly used worldwide as well as in the United States. Almost half of the U.S. national database already contains data for these loci. Finally, it has been suggested that the DYS391 locus be added to confirm amelogenin null alleles sometimes present in DNA profiles.[3]

In the past few years, Promega Corporation and Life Technologies have released several new next-generation STR multiplex kits that enable complete coverage of all of these additional loci plus the 13 U.S. core loci. These multiplex kits have been extensively tested in the National Institute of Standards and Technology (NIST) laboratory, allowing the probability of identity calculations to be made with different sets of loci and population statistics, including allele frequencies, observed alleles and genotypes, polymorphism information content (PIC) and heterozygosity values for each locus, to be determined with a standard set of unrelated U.S. population samples. With this information, it has been possible to thoroughly characterize these new STR loci beyond the original 13 CODIS core loci to determine the impact that this additional information will have on database searches.

A summary of these results, including STR locus population statistics for the new STR loci, will be shown in order to help assess the benefits of adding additional loci to the current 13 CODIS core loci.

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Forensic DNA, STR Multiplex Kits, New STR Loci

A91 UV-Visible Microspectrometer Parameters That Affect Spectral Quality, Reliability, and Power of Discrimination

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After attending this presentation, attendees will benefit by gaining a deeper understanding of how UV-visible microspectrophotometry (MSP) spectral data quality, reliability, and discrimination power are improved through testing and controlling critical instrumental parameters, specifically, attention to sample preparation and using polarized light when analyzing optically birefringent samples.

This presentation will impact the forensic science community by recommending guidelines for collection of high-quality spectral data and interpretations in ultraviolet-visable MSP analysis.

Microanalysis of transfer (trace) evidence involves the application of a microscope and microscopical techniques for the observation, collection, documentation, and analysis of micrometer-size particles. Microspectroscopy is the union of microscopy and spectroscopy for microanalysis. Microscopy is the art and science of creating, observing, recording, and interpretation of magnified images. Analytical spectroscopy is the science of studying the absorption, reflection, and emission of electromagnetic radiation to determine the structure and chemical composition of materials. Both microscopy and spectroscopy contribute specific scientific information about a material's structure and composition. Both scientific methodologies originate with optics and the interaction of radiant energy with matter. Microspectrophotometry is perhaps one of the oldest microanalytical techniques with roots traced to the microspectrograph in the mid-1800s when used by Henry Clifton Sorby in order to compare the transmission spectrum of light traversing different directions in crystals. Microspectrophotometry has various contrast methods. These techniques continue to be used today in forensic science laboratories for the characterization and comparison of naturally colored and dyed or pigmented transfer evidence such as architectural, automotive, and artistic paints, natural and synthetic fibers, color-dyed human and animal hairs, thin polymer films, and minerals.

This research reports the critically important role of factors such as: (1) using a research PLM with a circular rotating stage to precisely orient the sample; (2) controlling the orientation of polarizer in the condenser; (3) the quality of the spectrometer used to record transmittance; (4) importance of slide and cover slip; (5) using a refractive index oil that closely matches the refractive index of the specimen to minimize scattering and lensing effects; and, (6) the proper treatment of data, including spectral normalization, in order to determine real variance in the data. This research reports how wavelength accuracy was determined using a combination of mercury-argon and krypton line sources. Photometric accuracy was determined using a series of neutral density filters. The system's noise was determined by measuring root mean square (RMS) noise of the transmittance. The results of this research will be presented in a two-prong approach: instrumental parameters and sample-dependent factors.

Frequently, inherent instrument-induced polarization effects are not realized by the untrained user. Spectral data collected on fibers and films demonstrate that instrument-induced polarization may result in erroneous interpretations, false exclusions, and false inclusions based on changes to spectral features such as bathochromic or hypsochromic peak shifts, and hyperchromic or hypochromic intensity shifts. The results will also demonstrate that sample preparation and specimen orientation are critical to obtaining high-quality (high signal-to-noise) data. Specimens are generally not flat or parallel materials so physical characteristics such as cross-section morphology, varying levels of pigmentation, variations in dye uptake, and degree of delustrant will all effect the quantitative transmittance level, the quality of the spectral data, and the degree of spectral consistency. The results will demonstrate that proper data processing (normalization) will minimize and control sample variations due to sample thickness (pathlength) and sample concentration variations in the specimen.

In conclusion, the proper use of the microscope is critical to obtaining reproducible, high-quality, high-resolution data which is essential for optimum spectral differentiation. When the operator systematically controls as many instrument parameters as possible, the quality of the spectral data is improved, which results in a higher signal-to-noise and better spectral discrimination. The goal of this presentationis that the guidelines that will be presented will be considered for adoption by SWGMAT, ENFSI, and other forensic science technical groups.

Microspectroscopy, Trace Evidence, Criminalistics

A92 Utilizing Comprehensive Gas Chromatography Coupled to Fast-Scanning Time-of-Flight Mass Spectrometry for the Analysis of Forensically-Relevant Samples

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After attending this presentation, attendees will gain an understanding of the technique of comprehensive gas chromatography, or GCxGC, and how this technique can aid the forensic chemist for a variety of analyses including fire debris and drug analysis. Additionally, the benefit to the legal system will also be discussed, where the output from this technique may be more easily explainable and understandable to the court.

This presentation will impact the forensic science community by educating potential users of this technique as to its abilities, and how they might be used in a variety of analytical methods to provide greater data quality, and also easier comprehension to a non-scientific audience such as the legal system.

Comprehensive GCxGC is a relatively recent technique available to separations scientists. Largely credited to Phillips, this technique has recently become more user-friendly, robust, and accepted in the scientific literature.[1,2] Additionally, several systems are now available to potential users, which increases the potential for application to the field of forensic science.

This presentation will address the fundamentals and instrumentation for GCxGC. Specifically, the use of cryogenic modulation GCxGC coupled to Time-Of Flight Mass Spectrometry (TOF-MS) will be discussed, allowing for an increase in chromatographic peak capacity and sensitivity for many complex separations. This increase in peak capacity allows for higher data quality when analyzing complex samples. Through the examples of fire debris and drug analysis, this presentation will demonstrate that GCxGC-TOF-MS should be considered as a viable analytical technique in the forensic science field because: (1) it allows for the classification of compounds based upon chemical functionality, thus allowing for tentative peak assignment even in the absence of reference standards; (2) it allows for greater peak resolution which improves both qualitative id and quantification accuracy; (3) it allows for data display that is much more easily explained to members of the legal community who may not have years of analytical chemical education and experience; and, (4) it may allow for identification of marker compounds and other trace compounds that are obscured for a variety of reasons in more conventional analyses.

Specific examples of pharmaceuticals in wastewater samples and data from an arson case will be presented to briefly demonstrate the benefits of this technique. The analysis of drugs in wastewater may allow for spatial and temporal resolution of usage in a population, but the analysis conditions are difficult using conventional approaches. Many of the drug compounds and metabolites are at very low levels of concentration relative to the variety of co-extractable, or matrix, compounds that are also found in wastewater. If these matrix compounds are not resolved in the chromatographic analysis, they may either obscure the trace compounds or bias the mass spectrum such that detection becomes difficult. GCxGC-TOF-MS data will be shown in comparison to GC/MS data to demonstrate the utility of the technique.

Equally, fire debris samples present a difficult separation. Most potential accelerants are hydrocarbon-based distillation fractions, and contain a large number of individual compounds. In some cases (diesel and kerosene, for example), these materials can be difficult to distinguish from one another, especially once they have been weathered either through evaporative loss or through combustion. Again, the increased peak capacity and selectivity of GCxGC-TOF-MS will be compared to the more conventional GC/MS technique to demonstrate the benefits of the use of this technique to the forensic community.

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Fire Debris, Drug Analysis, GCxGC/TOF-MS

A93 Present Status of Gas Chromatographic Stationary Phases Being Used in Forensic Science

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After attending this presentation, attendees will have a better understanding of how the different stationary phases used in gas chromatography (GC) are being applied to separating various analytes encountered in forensic science laboratories.

This presentation will impact the forensic science community by providing information on the different gas chromatographic stationary phases available and how they are being utilized for the separation of analytes in different matrices found in forensic science.

GC is a separation technique that has been used in most analytical laboratories for over 50 years. Most crime laboratories, especially those performing toxicological and controlled substance analysis have at least one gas chromatograph in their laboratory. To date, there has not been a survey of GC users specifically targeted at the forensic sciences. *LCGC North America* magazine has published surveys in 1990, in 1995, and in 2003 of GC users in the chemical industry.[1-3] Several changes have occurred in the industry and in forensic science since these surveys. For example, many environmental laboratories, which at one time represented one-third of all analytical laboratories that used gas chromatographic columns, have been closed. In recent years, many pharmaceutical laboratories have also closed or consolidated resources, outsourcing much of their analytical work outside the United States. In addition, there are now more forensic laboratories performing GC and GC-mass spectrometry (GC/MS) than there were 20 years ago. In 1995, forensic laboratories only represented 2.6% of the laboratories surveyed and in 2003 the forensic laboratories representation increased to 6%.[2,3] The increased prevalence of gas chromatographic instrumentation in the crime laboratories is directly attributed to the reduced cost and availability of instrumentation, particularly the table-top gas chromatograph-mass spectrometer (mass detector). New manufacturers of gas chromatographic columns and new and improved column technology have also emerged, making a larger variety of stationary phases available.

With all of these changes taking place and new designer drugs challenging controlled substance and toxicology laboratories daily, it was thought that a survey of the use of gas chromatographic stationary phases for different applications would be of interest to the forensic community. Surveys were mailed to over 100 random selected crime laboratories and forensic scientists around the country. The results of these surveys will be presented. In addition to the surveys, the application of GC and GC/MS in recent proficiency tests was also investigated. Finally, the forensic science literature was searched from 2007–2012 to assess the use of different stationary phases, the technical specifications of the columns, and how each were applied to separate various analytes found in forensic samples, i.e., various drugs and toxins of interest in controlled substance and toxicological analysis, fire debris samples, pyrolysis GC, etc. Not surprisingly, preliminary results indicate that capillary columns are overwhelmingly being used in crime laboratories. Columns of 0.2 to 0.25- and 0.32mm i.d. in a range of lengths of 12 to 30-m lengths are the most popular. 100% Methylsilicone, 5% phenylmethylsilicone, polyethylene glycol, and 50% phenylmethylsilicone continue to be the most popular stationary phases.

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Forensic Science, Gas Chromatography, Stationary Phases

A94 Application of Raman Spectroscopy to the Forensic Analysis of Drugs, Controlled Substances, and Fibers

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After attending this presentation, attendees will learn of the application of Raman microscopy to drugs and fiber analysis.

This presentation will impact the forensic science community by providing methodology and technical details of using micro-Raman analysis of drugs, controlled substances, and fibers.

Raman spectroscopy and infrared spectroscopy are complementary techniques often used for the identification of compounds. Raman spectroscopy; however, offers several advantages over infrared spectroscopy. Raman spectroscopy is a light-scattering technique whereby light from a laser interacts with a sample producing scattered light of different wavelengths. The scattered light which is specific to a particular compound is funneled to a detector enabling chemical identification.

Raman analysis has been recognized to have potential for solving a wide variety of problems associated with forensic science. Early motivation was to identify substances/contaminants that appeared in crime scene evidence and manufactured products; however, it was quickly applied to all types of materials analysis.

Raman spectroscopy is very applicable in the field of forensic science. It uses a technique that offers a non-destructive and non-contact method of analysis. Only a small amount of sample is required and little or no sample preparation is necessary. It allows for trace analysis, and sampling can be done directly through transparent evidence bags and packaging such as glass and plastics. It covers a wide spectral range, from 1000cm^{-1} to 4000cm^{-1} making the technique ideal for the identification of both organic and inorganic substances, which includes drugs, pharmaceuticals, explosives, fibres, inks, paint, etc. Bulk analysis and screening of opaque materials such as powders is also possible. Raman spectroscopy also allows for the identification of the components of non-homogeneous samples and automated high-definition Raman mapped images can be obtained.

The purpose of this paper is to demonstrate some of the forensic applications of Raman spectroscopy. In particular, the capability of Raman spectroscopy to differentiate between drugs of similar structure will be demonstrated. That is, Raman spectroscopy has the capability of detecting even slight differences in the chemical composition of a drug and, therefore, plays a vital role in helping to determine when drugs have been illegally manufactured. In order to illustrate the abovementioned, spectra of the two main forms of cocaine (hydrochloride and base) will be highlighted in this paper as well as the ability to identify drugs in plastic bags/containers.

In an effort to aid law enforcement personnel and the public at large, investigations have been geared toward the ability of Raman spectroscopy to identify a variety of polymers used in fibers. This is very important as the presence of fibers at a crime scene has often been instrumental in the process of solving crime. “Fingerprints” of nylon 6, Kevlar, poly-styrene, PET, poly-propylene, and some others, along with different types of nylon (nylon 6, nylon 66, nylon 12, and others) will be presented.

Data will be collected using both a 633nm and a 785nm lasers. Comparison of the drugs and fibers will be done using similar wavelength lasers.

Raman spectra will be presented and method development including statistical analysis will be described. It will be shown that a search can provide quick identification of materials whose spectra have been collected in a library, or just matched to suspect material samples.

Drugs, Fibers, Raman

A95 An Investigation Into the Volatile Organic Compounds Released From Submerged Remains

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After attending this presentation, attendees will have a better understanding of the chemistry of death, also known as thanatochemistry, the volatile organic compounds (VOCs) that evolve from decomposing remains and how they are affected by water.

This presentation will impact the forensic science community by providing results on a new facet in the study of decomposition that includes the assessment of VOCs from submerged remains, under controlled conditions.

Depending upon the environmental conditions and surroundings of the body, the decomposition process starts immediately after death and initiates with autolysis. In this stage, intra- and extracellular enzymes present within the body cause molecules and cells to break down, leading to the second phase of the decomposition process known as the putrefactive stage. The degradation of cells that occurs during autolysis creates an ideal environment for anaerobic microorganisms to break down large molecules, such as carbohydrates, nucleic acids, proteins, and lipids, causing the release of gases, which range in functionality (e.g., amines, sulfur-containing, acids, alcohols, etc.), as well as discoloration and bloating.[1,2] Thereafter, active decay is said to begin. In this phase, the body is no longer bloating and the decomposition process continues with additional degradation of proteins and fat. The subsequent stage is advanced decay, which is where the body dries up and remnants (e.g., skin, cartilage, etc.) are present. This stage paves the way to the final phase of decomposition, skeletonization. It is at this point that only bones and hair remain.[3]

The decomposition process is highly influenced by the environment that surrounds the body. Factors, such as moisture, temperature, presence of scavengers, and oxygen availability, can alter the manner in which a body decomposes and, thus, the liberation of volatile organic compounds. Previous studies have evaluated the effects of soil on the evolution of VOCs from both humans and analogues.[3,4,5] However, little to no research has been conducted on the VOCs that evolve from submerged remains. According to Osterkamp,[6] the increased use of water-search canines emphasized the importance of thoroughly understanding the VOCs released from submerged remains to improve canine performance in locating drowned individuals. As previously mentioned, there are a variety of factors that can affect the manner in which a body decomposes and water is another important aspect to consider. The decomposition process of a body submerged in water can either be reduced or accelerated depending upon the type, temperature, pH, and flow rate of water.

This study used Headspace Solid-Phase Microextraction (HS-SPME) coupled to Gas Chromatography-Mass Spectrometry (GC/MS) to evaluate the volatile organic compounds that are released from decomposing submerged remains. Freshly killed human cadaver analogues were placed into water and allowed to decompose while subsequently being monitored at different time intervals to assess VOCs that were being released. The compounds detected ranged in functionality from acids to sulfur-containing; moreover, the type and abundances of compounds detected changed over time, which was expected since mammalian decomposition is a process and not a single event. The results obtained were then compared to a previous study that evaluated the VOCs that were released during the decomposition process of non-submerged human cadaver analogues. Differences, as well as similarities, in the compounds detected will be discussed in this presentation, as well as the impact that water has on the release of VOCs from decomposing submerged remains.

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Submerged Remains, Decomposition, Volatile Organic Compounds

A96 What Can Be Done to Save Criminalistics?

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The goals of this presentation are to: (1) raise awareness of the disparity between the potential of criminalistics to contribute to the justice system and its actualization; and, (2) to draw ideas and suggestions from the audience for overcoming the considerable obstacles to the realization of this potential.

This presentation will impact the forensic science community by pointing out ways in which the scientific potential of criminalistics can be enhanced to contribute to justice.

Perhaps, one would think the first question to be asked is: Is criminalistics worth saving? However, in order to address that question, there are some other questions that need to be answered. For example, what is criminalistics? What is its function? Does it contribute something of value? Do we have consensus on these questions? Let's throw out some thoughts for consideration. Before attempting to tackle the question of what it is, it should be sserted that I very strongly advocate that criminalistics is capable of contributing. Criminalistics is capable of establishing the "ground truth" in difficult criminal and civil investigations based on the physical evidence record. No other mode of investigation (eyewitnesses, interrogation, confession, etc.) has anything approaching this same powerful potential to offer. The alternatives are more error prone as well. Is this same potential realized in practice? Clearly, it is not. Why not? Can we enumerate the reasons?

Lets look at what's wrong; however, a general definition of Criminalistics should be provided first. Perhaps it is a definition which can be agreed upon using the AAFS structure as a model and starting point. We all hold some reasoning or ideas of why we are members of the Criminalistics section and not another. What are the attributes and foci of those of us in the Criminalistics Section that define us? Could it be said that we are scientists whose focus is applying scientific thinking, scientific knowledge, and scientific methods to the interpretation of physical evidence from suspects, victims, and crime scenes? How many of us engage in this practice in the broadest sense? How many of us, by choice or not, act more like technicians than scientists, and are restricted to testing samples in a more or less reactive manner? The anecdotal evidence I have is that most of us (worldwide) are not given the opportunity to formulate and address whole scientific questions. We are constrained to a reactive role where someone else, typically a nonscientist, poses the scientific questions to be addressed in a given investigation, and therefore, we are unable to provide the most value. It may seem obvious to us that a scientist is the best qualified to pose a scientific question. Strangely, it doesn't seem as though this view is widely shared, or perhaps even thought about. If this trend toward the reactive role were to continue, could we not be replaced by automatons and easy-to-operate, field-deployable instruments? This would be the end of criminalistics. Whether appreciated or not, it would be the end of scientific input to investigations. On the surface, it would appear to be cost-effective, but investigations and valid evidence interpretation at the adjudicative stage would suffer immeasurably. Is this alarmist? I don't think so.

Now that we may have somewhat of a consensus on what criminalistics is, and perhaps a consensus on what is wrong with our field, a discussion of remedies can begin.

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Criminalistics, Science, Investigation

A97 The Laboratory Report Project Part 1: Content Analysis of Laboratory Reports

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After attending this presentation, attendees will understand how content in typical forensic science laboratory reports are submitted as the result of the analysis of evidence.

This presentation will impact the forensic science community by informing attendeesof the status of laboratory reports today in various disciplines and jurisdictions so that crime lab directors can compare their practices with those of the community at large.

This project is a result of recommendation #2 of the National Academy of Sciences (NAS) Report on Forensic Science—"that forensic science laboratory reports follow a standard, scientific format". Testimony before the NAS Forensic Science Committee indicated that too many laboratory reports were little more than "certificates of analysis" that contained only descriptive information about the evidence and the results of the analysis, without any information about the methods and procedures used, and little or no presentation of the data and how the conclusions were reached. It was felt by the Committee that this type of report wasn't reflective of the scientific nature of forensic science analysis. Before developing such a standard, it is important to know what is currently being submitted in the way of lab reports. With the help of the American Society of Crime Lab Directors, forensic science lab directors were asked to submit specimens of redacted laboratory reports and/or report format templates in several disciplines including drugs, DNA, fingerprints, questioned documents, toxicology, and trace evidence. The only demographic information that was sought was the governmental level of the laboratory (federal, state, regional, local). More than 400 laboratory reports were ultimately received. The reports were first categorized by laboratory type and then by report type. A spreadsheet was created that listed all of the sections of a model laboratory report that would be expected: demographics of submission, request for analysis, description of the evidence, methods and materials of analysis, analytical procedures, results (data), discussion of the results, and conclusions including limitations and sources as well as magnitudes of possible errors or uncertainties in the conclusions. Each of the laboratory reports was then analyzed to determine which of these sections were present in that report. After this was completed, each type of report (drugs, DNA, etc.) was examined separately to determine what sections were most commonly found and what sections were not usually present. Determinations were made to see if particular types of laboratory reports were more complete (in the sense of having the most sections) than other types of reports. The data were then analyzed to determine if there were significant differences between laboratory reports generated by federal, state, regional, or local laboratories, and, if possible, whether differences existed between public and private laboratories. On the basis of the data collected in this study, attempts will be made to determine if it is possible or desirable to develop a model template that all laboratory reports, regardless of jurisdiction and regardless of report type, or does it make more sense to develop a set

of templates that reflect the type of analysis being done; that is a different type of report for a drug case from a fingerprint case. Consideration will be given to the needs of prosecutors and defense attorneys to be able to quickly determine the results of a forensic science analysis for the purposes of decision making in the adjudicative process – this might take the form of an "executive summary" of the report.

Laboratory Report, NAS, Model Lab Report

A98 The Laboratory Report Project Part 2: Proposed Standards for Forensic Laboratory Reports

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After attending this presentation, attendees will be able to discuss the proposed criteria for forensic science laboratory reports that are being offered by various organizations, including the American Society for Testing and Materials, the American Bar Association, the American Society of Crime Lab Directors, and specific disciplines within the forensic science community, such as questioned document examiners and firearms/tool marks examiners.

This presentation will impact the forensic science community by presenting the information needed to reach consensus on how scientific lab reports should be compiled.

This presentation is the second of a two-part study of laboratory reports that are issued by forensic science laboratories at the conclusion of their analysis of various types of evidence. The first part of this study was the collection of more than 400 redacted forensic science laboratory reports from members of the American Society of Crime Lab Directors and then content analysis to determine what is actually present in forensic science laboratory reports. This project included reports from several sections of laboratories including drugs, toxicology, DNA, fingerprints, documents, etc. and specimens were solicited from federal, statewide, regional, and local laboratories. The impetus for the study came from the Forensic Science Committee of the National Academy of Sciences that issued its report in 2009. One of the recommendations of the Committee was that forensic scientists adopt a standard laboratory format that presents analytical findings in a rigorous scientific format. Partly as a result of the NAS Report, several organizations have developed models for forensic science laboratory reports. Some of these organizations are in the area of general science, others in the criminal justice system, and still others in forensic science itself. This presentation will relate the major features of all of these organizational recommendations. The goal will be to determine if there is a consensus among organizations as to the content of laboratory reports. The overall study will address the issues of whether there ought to be a single model for all of forensic science, regardless of the type of laboratory report or the jurisdiction of the reporting laboratory, or would it be better to have standard formats for each discipline within forensic science. Whatever standards are ultimately adopted, if any, they must meet the needs of science and criminal justice. A good scientific laboratory report is complete and transparent. It presents all processes and procedures, data, results, conclusions, and limitations including sources and magnitudes of possible error. The current practice in forensic science is for laboratory reports to not contain all of these sections, especially the raw data. Forensic science laboratories feel that this requirement is unduly burdensome, it can be handled by discovery, and all of this detail is not needed, appreciated, or read by attorneys or judges. This project will explore these tradeoffs and possible solutions. The NAS Committee felt strongly that laboratory reports must be scientifically rigorous and complete if forensic science is to be recognized as legitimate science. Discovery is an imperfect means for getting at all of the facts and data present in many forensic science cases.

Laboratory Report, NAS, Model Lab Report

A99 Is There a Need for a (Forensic) Science Ombudsman?

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The goals of this presentation are to learn the role of an ombudsman in an organization, the suggested role of a scientific ombudsman, and the benefits of such a position in forensic laboratories regarding quality, ethics, operations, and safety.

This presentation will impact the forensic science community by offering a novel approach to the mitigation of inevitable conflicts that arise in organizations and, with the creation of a scientific ombudsman, could stop or even prevent some failures that are all too common in the profession. The impact on the profession could be significant.

Conflicts arise within any organization; the role of an ombudsman is to act as a neutral, independent arbiter and mitigator of procedural or ethical conflicts. An ombudsman operates within an organization but outside the traditional hierarchy, typically answering only to the highest authority in the hierarchy. As inside "outsiders," ombudsmen act as informal, impartial sources trained in conflict resolution, providing safe places to share concerns, facilitate early resolutions, and help answer complex or sensitive questions.

Repeated and persistent failures of process and quality control at various forensic science laboratories and service providers suggest the need for a Scientific Ombudsman (SO), one that would not only address the standard questions of conflict in an organization, but also issues of scientific conflict, such as method development, process and protocol application and execution, training and competency testing, proficiency testing, scientific ethics, testimony, and reporting. Lapses in the scientific systems within laboratories, such as North Carolina, Nassau (NY), Houston (TX), and St. Paul (MN) point to the need for internal corrective mechanisms that are outside the traditional hierarchy of laboratory management, especially for those working within a police culture which operates under different mores and norms than would a scientific one. Organizations have already adopted the role of the ombudsman, such as in North Carolina; while laudable, there is a persistent need for an ombudsman whose background includes science and particularly forensic science. For example, in a recent case, this exchange occurred.

> Attorney: "You dont, in your lab, have a lot of the basic minimum standards in place?"
>
> Scientist: "I guess I don't know what the minimum standards are."

At first glance, this may seem to be a quality issue, but if the lack of standardization or even the awareness of it is institutionalized in a laboratory, the concerned scientist cannot turn to management. Management is the source of the problem. Another example would be where laboratory policy requires an inconclusive reporting statement when evidence from the person in question is lacking, causing a scientist to consider this an exclusion; the supervisor rejected the scientist's concerns.

The role of the SO would be similar to a standard ombudsman but with important differences. Any ombudsman is neutral, independent, confidential, and informal. Neutrality means that everyone is treated with equal respect, regardless of status or rank. The ombudsman has no stake in the outcome but rather acts as an advocate for an equitable process and outcome while navigating potential conflicts of interest. Independence means that the ombudsmans position is located in the hierarchy such that it reports directly only to the highest levels of management. Confidentiality; however, is necessary to help resolve conflicts at the lowest levels possible. The only time an ombudsman would release any information without the approval of the complainant would be instances of imminent threat or harm. Finally, an ombudsman is informal: no records (other than statistics) are kept and the ombudsman does not participate in any formal judgment processes.

The SOs role would be the same as any other ombudsmans with several important additions. Beyond the skills and training of a typical ombudsman (e.g., alternative conflict resolution methods), the SO needs to have a science education and career experience, a deep understanding of forensic issues, and training in ethics, quality, and

accreditation; additionally, the SO would need a deep understanding of the criminal justice system, the constitution, and the rights of the accused. The benefits to a forensic laboratory of having an SO include increased productivity, improved management, cost savings in personnel (reduction in lawsuits, turnover, and union issues), cost reductions in legal staff, and other benefits, such as improved morale and reduced illegal or unethical behavior. Many of these benefits involve significant cost savings for the laboratory. The SO is successful if the office is seen as safe, accessible, and credible; employees help themselves to resolve issues; and the management has feedback to improve the organization. The SO is in a unique position to identify and communicate new opportunities and innovations.

The concept of a scientific ombudsman has the potential to improve forensic science operations, effectiveness, and professionalism.

Ombudsman, Negotiations, Professionalism

A100 Tacit Knowledge, Deliberate Practice, and the Development of Expertise in the Forensic Sciences

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After attending this presentation, attendees will understand the concepts of tacit knowledge and deliberate practice and the role these concepts play in the development of expertise in forensic science.

This presentation will impact the forensic science community by emphasizing the need for forensic examiners to develop personal programs of deliberate practice (research, continuing education, and critical case review) in order to attain the highest level of professional expertise.

This paper reviews recent research on the development of expertise (defined as a high level of performance in a given field) and examines the ramifications of this research for graduate education in the forensic sciences. It will also examine the nature of the scientific knowledge, which students must absorb and then look at the means by which this knowledge is presented to students. The 2009 National Academy of Sciences Report, "*Strengthening Forensic Science in the United States: A Path Forward"* stated: Forensic science examiners need additional training in the principles, practices, and contexts of scientific methodology, as well as in the distinctive features of their specialty. Training should move well beyond intern-like transmittal of practices to teaching that is based on scientifically valid principles.

While no one would contest the value of more education in scientific methodology, the denigration of intern-like training is problematic. According to philosopher Michael Polyani, sociologist Harry Collins, and others, all scientific fields have a component of tacit knowledge—knowledge obtained by deep immersion in groups that possess it. Tacit knowledge comprises knowledge that cannot be gained by reading scientific texts or even by reviewing the peer-reviewed research literature in a discipline. Moreover, tacit knowledge is required for the development of expertise in a scientific discipline. Intern-like transmittal of practices within a scientific discipline is a legitimate mechanism for the conveyance of tacit knowledge (as is on-the-job training in a forensic science laboratory). Doctoral programs in the experimental sciences have long consisted of intensive, intern-like exposure to the practices of the particular field of study. Such exposure is an essential antecedent to conducting doctoral level research.

Extensive research by K. Anders Ericsson and his colleagues has shown that high levels of performance in fields such as athletics, music, and science are the result of long periods of what Ericsson has termed deliberate practice. Deliberate practice means not merely repeating the same tennis swings or pieces of music over and over again. Rather it means pushing the envelope, tackling more and more difficult activities. Deliberate practice also involves a considerable degree of critical self-reflection. According to Ericsson, approximately ten years or 10,000 hours of deliberate practice are necessary for the attainment of true expertise in a field.

The typical graduate student in the forensic sciences begins his or her graduate degree program after completion of four years of study. Only a small part of undergraduate study would qualify as deliberate practice. Even after the student obtains his or her graduate degree in forensic science and moves into a forensic laboratory (perhaps completing a short on-the-job training program), the student is well short of the ten years or 10,000 hours of deliberate practice required to attain true expertise. While reading the scientific literature and attending workshops allow the bench forensic scientist to remain current in his or her field, such activities do not constitute deliberate practice. Moreover, case work in general does not constitute what Ericsson means by deliberate practice. Case work in many forensic science fields is routine and does not challenge the forensic examiner to extend his or her skill set.

Graduate degree programs in forensic science should have three goals: reinforcing students' grasp of scientific methodology, conveying tacit knowledge in the students' fields of concentration, and developing habits of deliberate practice in students. Faculty in graduate forensic science degree programs must possess the tacit knowledge in their respective fields of expertise and may need their own programs of deliberate practice to achieve true expertise. The habits of deliberate practice that graduates can carry into their further careers could include ongoing forensic research, and critical reviews of completed cases. Other approaches to continuing deliberate practice will also be discussed.

Tacit Knowledge, Deliberate Practice, Expertise

A101 Results From the 2009 BJS Census of Publicly Funded Forensic Crime Laboratories

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After attending this presentation, attendees will learn about the state of U.S. publicly funded forensic crime laboratories in 2009 as captured by a national census, which was designed to collect data on a wide range of public laboratory characteristics. Also, the federal agency that commissioned the collection, the research organization that performed the data collection, and the process that each organization undertook to finalize the survey, collect, and analyze the data will be discussed.

This presentation will impact the forensic science community by presenting national-level estimates of public laboratory workload, budget and operations as reported for the year 2009, a one-of-a-kind data collection effort that may influence state and federal legislation, help shape forensic science policy, and aid those seeking to conduct research on the local, state, and federal forensic science systems.

Publicly funded forensic crime labs are a vital component of the criminal justice system, receiving millions of pieces of evidence from law enforcement investigations each year. Within the criminal justice system, there is an ever-increasing reliance on forensic evidence, as well as increasing concern over the role of the nation's forensic crime labs in processing the evidence, issuing laboratory reports, and their subsequent use in legal proceedings.

The Bureau of Justice Statistics' (BJS) Census of Publicly Funded Forensic Crime Laboratories is a recurring data collection that provides a national picture of the services and resources devoted to forensic laboratory activities across the country. This data has been used to help inform policy making and planning at all levels of government. The 2002 and 2005 censuses documented the backlogs in requests for a wide range of forensic services, including controlled substance identification, firearm/tool mark analysis, and latent fingerprint examination. Previously, public attention had been focused almost exclusively on DNA backlogs. A third census was fielded in 2010 and 2011 to capture

detailed data on the workload and operations of the more than 400 federal, state, and local publicly funded forensic crime labs operating in 2009.

This presentation will examine the forensic services provided by publicly funded crime labs during 2009 and identify the evidence areas that account for the largest portion of the national backlog. It will also assess changes since 2002 in the budgets, staffing levels, and quality assurances in forensic crime labs, including lab accreditation, proficiency testing, and the resources devoted to research. The presentation will also examine the uses of advanced technologies to process evidence received from criminal investigations and laboratory information management systems to organize and document laboratory operational and experimental data.

The 2009 census achieved a response rate of ninety-seven percent, the highest in the history of this data collection. Therefore, the statistics produced from these data, and reported in this presentation, provide the most nationally representative information to date.

Census, Public Laboratories, Workload

A102 The Challenges of Translating Forensic Science Research Into Practice: Resources, Backlogs, and Accreditation

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After attending this presentation, attendees will have discussed major challenges of translating reseach into practice for forensic laboratories, which are limited resources, increased backlogs, and stringent requirements for accreditation.

This presentation will impact the forensic science community by attempting to dissect these challenging issues with an emphasis on the fundamentally important need for research, but even more vital, translating research into practice.

In 2009, the National Research Council, an arm of the National Academy of Sciences (NAS), published, *Strengthening Forensic Science in the United States: A Path Forward* and stated the following: *Forensic science often produces valuable evidence that can be used to successfully prosecute and convict criminals, as well as exonerate the innocent. Based on technological innovations and the evolution of the field in the past several decades, forensic scientists, as a whole, have continued to improve knowledge and better understand methods and practices. After years of general acceptance about theories and fundamental concepts, the criminal justice system is now demanding scientists to demonstrate that the methods and practices employed in various forensic disciplines are based on accurate, reliable, and valid testing.*

Following the NAS Report, there has been an increase in forensic science research throughout the nation and this has been evidenced by a significant increase in funding by the National Institute of Justice (NIJ) for research, development, and evaluation. From 2009 through 2011, NIJ has funded 174 projects for a total funding of $71,280,619, which has resulted in 122 publications, 264 presentations, and 28 final technical reports. Approximately one-third of the total funding since 2009 has been allocated to basic and fundamental research, but the far majority of funding in forensic science still focuses on applied research and new technology. Analytical instruments, software packages for data processing, and database technology in the forensic sciences have evolved at a significant pace over the past two decades. Although forensic laboratories throughout the United States continue to make progress analyzing evidence efficiently and effectively, backlogs continue to be a major challenge for many forensic service providers. Research in the forensic sciences is often perceived solely as a means to strengthen the underlying science, but research, conducted in the appropriate capacity, can be the impetus to increased quality and efficiency. The 2009 Census of Publicly Funded Forensic Crime Laboratories was released in August 2012 showing that resources dedicated to research diminished significantly from 2002 until 2009. With this reduction in resources, it then becomes very difficult to test and implement advanced technology.

What is the underlying cause of these ever-increasing struggles that forensic laboratories must face? Is it lack of resources? Perhaps the stringent requirements necessary to achieve and maintain accreditation, which in effect, reduce efficiency? Or, are the ever-increasing backlogs prohibiting laboratories from embracing new technology? Should laboratories invest more in research as a means to become more efficient? Technology transfer can be a daunting task for any laboratory in terms of resources, and the question often arises whether multiple labs should be conducting tests on materials that are not frequently submitted and require expensive instrumentation. Is outsourcing a viable option for non-routine analysis? This presentation will attempt to dissect these challenging issues with an emphasis on the fundamentally important need for research, but even more vital, translating research into practice.

NAS, Research, Backlogs

A103 Certification and Accreditation: Useful Tools to Work Toward the International Standardization of Forensic Laboratories

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After attending this presentation, attendees will learn a different approach to design certification and accreditation processes of forensic laboratories, which determine complete control of all processes involved, and to fully document each step of the process and reach a reliable and reproducible conclusion.

This presentation will impact the forensic science community by presenting a new idea for revamping internal procedures of forensic laboratories, which use certification and accreditation processes to lower human errors. The ultimate objective is to set the basis for international standardization of procedures.

At the end of 2009, the European Union fixed the rules of the exchange of fingerprint and DNA evidence through the member States. From November 2015, only ISO 17025 accredited laboratories will be allowed to operate in an international context. The current situation across Europe could suggest a successful extension to all laboratories, operating in a national as well as international level. There is the need to create a common basis within the forensic community in order to be able to share not only the results of the analysis, but also the values. The definition of a common set of methods and contents is also highly recommended. Moving from this starting point, a deep analysis of methods and procedures currently used in western countries was carried out with respect to latent fingerprint laboratories, in order to properly model an overall procedure which starts from an item seized at the crime scene and ends in court with an individualization statement, expressed by a forensic expert.

This lecture presents the key factors for the successful accreditation from different perspectives: agencies, laboratory managers, and forensic experts. Initially the most effective way to establish a robust quality system in the forensic laboratory will be discussed. Even if not mandatory, implementing ISO 9001:2008 within the structure, as the preliminary step toward accreditation, is suggested. In a fully certified laboratory, the effort to reach the accreditation causes a minor impact when compared to the scenario where the agency looks for the accreditation ISO 17025 as the only goal.

Later, the focus will be driven on the documentation phase. The results of this phase are crucial for the accomplishment of successful accreditation. The perception of the accreditation process varies with the perspective of the agency, the management, and the practitioners. Documentation, if not properly designed, could cause a backlog increase and operators could start to perceive the accreditation process as an useless legal requirement.

To be able to turn the negative feeling, the implementation of an integral computer-aided documentation system, designed and

customized on the specific human resources and workflow of the single agency, may resolve all disputes on certification and accreditation processes.

The abovementioned concepts clarify that accreditation and certification are directly related to the reproducibility of the measure, but ISO guidelines do not suggest and/or require that the forensic laboratory choose between alternative possible processes.

The accreditation process could be used to critically revise all the internal procedures. The determination of the most reliable techniques, according to the most recent finding of the scientific research in the specific branch, is an important step forward standardization and human error management.

The forensic community must determine the minimum requirements for the forensic laboratories in terms of logistics, instruments, analytical procedures, personnel education level, training, competency, and proficiency testing with professional forensic organizations playing a role of paramount importance to accomplish this task.

Standardization, Accreditation, Certification

A104 High Resolution Fourier Transform Spectroscopy for the Discrimination of Lab Grade Explosive Precursors and Their Shop-Bought Equivalents

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After attending this presentation, attendees will have an understanding of the application of high resolution Fourier Transform infrared spectroscopy for the characterization and discrimination of explosives precursors and how this can be used to inform and develop explosives detection systems.

This presentation will impact the forensic science community by demonstrating the suitability of high resolution Fourier Transform mid-infrared spectroscopy for the discrimination of different brands of shop-bought materials which have the potential to be used as explosive precursors. The work will also explore the ability of high resolution Fourier Transform mid-infrared spectroscopy and chemometric analysis of the resultant data to distinguish shop-bought materials from each other and from their lab grade counterparts.

The development of accurate and sensitive explosives detection systems is an area of significant and substantial growth. The challenges facing the development of such systems are diverse, and, as a result, there are multiple approaches to solving them. However, one factor is constant through these approaches: in order to detect a compound; it must first be characterized in order to identify distinguishing features that can then be targets for detection.

While many explosives detection systems provide the ability to detect an explosive compound when part of an improvised explosive device, another approach is to further expand the capacity to detect explosive precursors as a means of identifying sites of illicit explosives manufacture, or those involved with the production of explosive materials. This has the potential to continue to prevent the production of explosive devices, and identify the bomb makers. Detecting the manufacture of explosive devices prior to completion has the demonstrated and significant benefit of making seized materials safer for the security services to handle and greatly reduces the risk to the public. An increased knowledge of explosive precursor materials would also continue and strengthen the existing understanding and detection of explosive devices as the materials involved will affect the breakdown of the explosive material and may be detectable as this occurs. For example, a hydrogen peroxide-based explosive such as Triactetone Triperoxide (TATP) will emit hydrogen peroxide vapor which can then be detected.

With the increase in the use of homemade explosive devices, there is continued desire to characterize the precursors of these materials. Due to the nature of these explosives, many of the precursor materials are available “off the shelf” and have many legitimate uses. However, the majority of these “off the shelf” chemicals will not be in a pure form, with various additives present to aid in the materials legitimate use. For example, acetone in nail polish removers is combined with perfumes, emulsifiers, emollients, aversive agents, and colorings. This work investigates whether it was possible to discriminate both between lab grade chemicals and their “off the shelf” counterparts, and between different brands of “off the shelf” materials using high resolution Fourier Transform spectroscopy and statistical analysis.

Previous work has demonstrated that high resolution Fourier Transform mid-infrared spectroscopy paired with basic data analysis is a useful tool for the characterization of explosives and explosive precursors. While the information gathered via this technique is a useful resource in its own right, it is also able to directly inform the development of a quantum cascade laser-based explosives detection system.

High resolution Fourier Transform mid-infrared spectroscopy should be particularly suited to the task of discrimination, as the increased resolution allows the system to be capable of resolving minute spectral features. In addition, while the instrumentation is able to look at minute details, it is also able to cover the whole mid-infrared region so the technique can be considered information rich. The impact of the information produced with a spectrum can then be enhanced by statistical analysis of the data.

This presentation reports the result of the comparison of lab grade liquid explosive precursors with “off the shelf” materials. The comparison and discrimination is made both by visual comparison of the spectra produced and also through statistical analysis of the data.

Explosives Detection, IR Spectroscopy, Statistics

A105 The Overlooked Potential of the Absorption of the Explosive Component 2,4-Dinitrotoluene by Disposable Gloves: Extraction and Detection

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After attending this presentation, attendees will understand how vapors from volatile compounds found in explosives can be absorbed by, and later extracted from, polymers and detected using chromatographic methods. The extraction of the explosives from two polymers will be described and detection of the explosive will be illustrated using High Performance Liquid Chromatography (HPLC). HPLC will also be used for the measurement of the kinetic parameters that determine how long an explosive component resides in the polymers.

This presentation will impact the forensic science community by showing how the results of the study indicate that both the nitrile rubber and latex rubber gloves absorb DNT at readily detectable levels. In the experiments reported here, the half-life of the DNT in nitrile rubber was measured to be 15.9 days. In the experiments, the DNT was detectable (HPLC with UV/VIS detection) in nitrile rubber samples 35 days after removing the rubber samples from a container that also held a small open vial containing solid DNT. The results indicate the potential use of the analysis of gloves to support their prior proximity and exposure to explosives. The period of time that the compound will remain detectable in the rubber will depend on the time it is exposed to the compound, the nature of the exposure (e.g., closed or open environment), the environmental conditions once the rubber is no longer exposed to a DNT source, and the detection method (the use of a gas chromatograph/mass spectrometer will extend the detection period).

The absorption of volatile compounds by specific materials is well documented, and is the basis of Solid Phase Micro Extraction (SPME). In SPME, a fiber coated with a specific absorbent is exposed in a container holding a sample, and the volatile compounds released by the

sample are absorbed by the material on the fiber. In this approach, the absorbed compounds concentrated on the fiber are isolated from the bulk of the sample. The absorbed compounds are released from the fiber later for analysis by heating the fiber. Polymeric materials found at crime scenes that possess the innate ability to absorb a volatile compound have apparently not been studied with respect to their potential to absorb volatile evidence. The hypothesis of this study is that specific polymers, for example, those that are found in disposable gloves, have the ability to absorb and retain explosive components which can be extracted later and detected.

In this study, the ability of latex and nitrile rubber polymers to absorb the vapors of 2,4-dinitrotoluene (DNT; a component in several explosive formulations) was studied. The extraction conditions were developed, and the half-life of the DNT in glove samples was determined. The use of DNT is uncommon in consumer products; aside from explosives, its primary use is to synthesize the precursor to azo dyes and polyurethane, both of which are found in consumer and industrial products. The DNT is used to synthesis toluene diamine and two or more synthetic steps later (depending on the product), the toluene diamine is converted to an azo dye or polyurethane. DNT has been reported as an impurity in precursors to polyurethane, and it is present as an environmental contaminant in soil and water in locations where DNT has been produced for the uses stated above.

The results of the study indicate that both the nitrile rubber and latex rubber gloves absorb DNT at readily detectable levels. In the experiments reported here, the half-life of the DNT in nitrile rubber was measured to be 15.9 days. In the experiments, the DNT was detectable (HPLC with UV/VIS detection) in nitrile rubber samples 35 days after removing the rubber samples from a container that also held a small open vial containing solid DNT. The results indicate the potential use of the analysis of gloves to support their prior proximity and exposure to explosives. The period of time that the compound will remain detectable in the rubber will depend on the time it is exposed to the compound, the nature of the exposure (e.g., closed or open environment), the environmental conditions once the rubber is no longer exposed to a DNT source, and the detection method (the use of a gas chromatograph/mass spectrometer will extend the detection period).

The principle established by the presented results will impact the forensic community by alerting the community to the potential use of polymers as evidence when volatile compounds are relevant to the case.

Explosive Detection, Explosive Vaporization, Explosive Absorption

A106 Hydrophobic and Hydrophilic Ionic Liquid Mixtures Used for Explosive Analytes

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After attending this presentation, attendees will have a general understanding of the potential applications afforded by mixtures of hydrophobic and hydrophilic ionic liquids, specifically their contributions to improved rate, specificity, and yield with forensic sampling of explosive analytes.

This presentation will impact the forensic science community by leading to the identification of homogenous ionic liquid mixtures that are able to collect a wide range of analytes of drugs and explosives, which is important to the forensic and chemical investigative community in ensuring relative ease and quickness when collecting field evidence.

Within the last decade, ionic liquids have become an increasingly popular topic within all disciplines of science because their unique physical and chemical properties have sparked interest in potential applications and innovative developments. Ionic liquids (ILs) are low-melting salts in their liquid state which are completely comprised of cations and anions. The development of room-temperature ILs in the early 1990s by chemists Wilkes and Zaworotko opened up a gateway toward more specified properties and functional possibilities, most notably advantages such as negligible vapor pressure, extreme thermal stability, customizable tailorability, and recyclability.[1] Especially with today's push for the Green Chemistry movement, it has become a higher priority to reduce or even eliminate the use of hazardous substances within chemical processes. Therefore the utilization of ionic liquids as a replacement for common solvents has been the focus of much research in recent years.[2,3] Depending on the specific ionic liquid, characteristics can range according to hydrophobicity, hydrophilicity, visoscity, conductivity, solubility, and electrochemical window.[3] With over 1,000 ionic liquids reported in literature today, there may be about 10^{18} possible cation/anion combinations—the properties and application possibilities are endless. The purpose of this study is to create homogenous ionic liquid mixtures consisting of a hydrophobic and hydrophilic component in an effort to maximize the range of analytes to be collected, retained, and analyzed quantitatively and qualitatively.

In investigative laboratories, specifically those pertaining to forensic sampling, it is in one's best interest to identify an optimum solvent for a group of compounds, i.e., one solvent for drugs and another for explosives; however, when examiners are working within the field, they are not able to distinguish one from another. For example, if an investigator comes across an unknown white powder, he is unable to tell whether it is cocaine, Triactetone Triperoxide (TATP), or simply powdered sugar. By using an essentially universal solvent, not only will the field agents reduce the amount of items they must carry onto the field, but they also minimize the risk of destroying the sample by using the wrong solvent and, due to the negligible vapor pressure, preserve the sample for analysis.

The approach of this study included tests for homogenous mixtures, solubility tests with four different explosives (TNT, Compound B, RDX, and PETN), which were then analyzed via Direct Analysis in Real Time-Mass Spectrometry (DART®-MS). It is common to have difficulty mixing molecular solvents, but it is considerably more complex when dealing with ionic liquids (salts) since each mixture could result in a binary mixture composed of three or four components as each salt must have an anion and a cation. In order to reduce unnecessary complexity, two ionic liquids with a common ion were selected for the homogenous tests. Out of the 27 mixtures tested, 17 were determined to be homogenous. Solubility tests with TNT were then conducted on the homogenous mixtures, in which six mixtures showed an immediate color change from clear and colorless to a dark purple which turned into a deep blood-red color over time. It is interesting to note that all six mixtures contained the EMIM-BF_4 ion. From previous studies, this drastic color change is suspected to be the cause of a charge transfer complex which reacts only with the TNT molecule. Nine of the remaining homogenous mixtures showed a slight or delayed color change. When the initial six mixtures, containing the EMIM-BF_4 ion, were tested with the other explosives, Compound B and RDX were deemed soluble, whereas PETN was considered only partially soluble or had limited solubility. Compound B was the only other explosive to display a color change within the mixtures, which is expected because it contains 40% TNT and 60% RDX. These samples were characterized via DART-MS, which confirmed the presence of TNT, RDX, and PETN within seconds. It is important to note that all analyte peaks were clean and there was no matrix interference from the collection swab, suggesting that the ionic liquid mixtures were able to not only retain the analyte, but also preserve the swab during analysis. This is an extremely important finding because this shows great potential for improved rate, specificity, and yield for forensic sampling.

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.

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Ionic Liquids, Explosive, Drugs

A107 The Influence of Acid Catalysts on Triacetone Triperoxide (TATP) Crystal Morphology

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Attendees of this presentation will learn about the different crystal morphologies formed as a result of using different acid catalysts during the synthesis of Triactetone Triperoxide (TATP).

This presentation will impact the forensic science community by revealing the considerable difference among crystals depending on the choice of acid, which may contribute to an investigation when the precursors are not known.

The use of improvised explosives for criminal, domestic and international terrorism is an ever-increasing problem. Methods for homemade manufacture of these non-military and non-industrial explosives often require little-to-no background in chemistry. TATP, a relatively exotic explosive, is currently receiving considerable attention because of this ease of procurement of starting materials and very simple synthesis reaction. First documented by Wolfenstein, the synthesis of TATP has been the subject of interest and research by scientists and hobbyists for over a century.[1] The two primary starting materials are easily available in relatively pure form from hardware stores, home improvement centers, and pharmacies, as well as in impure form when mixed with other compounds to create several different commercial products (e.g., bleaching agents, nail polish remover, drain cleaners, and many others). The "impurities" in these commercial products have been successfully studied and analyzed for carry-over into synthesized TATP by several instrumental methods.[2,3] Studies using different acid catalysts and their affect on the finished product have also been performed using instrumental methods.[4,5]

TATP is a powerful explosive but other undesirable properties exclude it as a viable explosive for military, commercial, or industrial use. In addition to its high vapor pressure, resulting in very rapid sublimation even at room temperature, it is far too sensitive to shock, impact, friction, and temperature for it to play a practical role in legitimate use, but these properties haven't discouraged its use in terror bombings (and bombing attempts) worldwide during its resurgence over the past 30 years. The dangers involved in the manufacture and handling of TATP have claimed the lives of many would-be "chemists;" in fact, the material is nicknamed the "Mother of Satan" by terrorist bomb makers due to its deadly instability and unpredictability.

The research presented here builds on previous work detailing the optical properties of two identified TATP polymorphs, and a preliminary study by Miller examining the crystal morphology of TATP synthesized using different acid catalysts.[6,7] This presentation expands on the work of Miller by further detailing the potential linkage between TATP crystal morphology and the specific acid catalyst used during its synthesis. The reagent-grade catalysts used for this study include sulfuric acid, hydrochloric acid, nitric acid, and phosphoric acid. The other parameters of the manufacturing process, including reaction temperature, number of times the precipitate was washed, and the temperature of the sublimation and recrystallization process, were kept identical. After synthesis and subsequent washing of the precipitate, the recrystallized crystal morphologies were clearly distinguishable from each other depending on which of the four acid catalysts was used during manufacture. Crystals catalyzed with sulfuric acid were almost cubic, those with hydrochloric acid were long needles, those with nitric acid were clumps of short needles, and those with phosphoric acid were rosettes. The results from these experiments provide a foundation in which a potential linkage between the acid catalyst used for TATP manufacture and the morphology of the synthesized crystals can be established. This information may potentially help as an investigative lead during initial phases of a bombing event when attempting to determine provenance of TATP samples recovered as evidence.

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TATP, Crystal Morphology, Microscopy

A108 Raman Microspectroscopy and Advanced Statistics for Detection and Characterization of Gunshot Residue (GSR)

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The goal of this presentation is to describe the development of a novel and alternative method for Gunshot Residue (GSR) analysis.[1] After attending this presentation, attendees will have a better understanding of recent advancements of this application of Raman microspectroscopy for GSR analysis, identification, and discrimination. The implementation of advanced statistics to differentiate experimental Raman spectra collected from non-equivalent GSR samples will be discussed.

This presentation will impact the forensic science community by having the potential to greatly impact the accuracy and effectiveness of shooting incident investigations.

Raman spectroscopy has numerous applications in forensic chemistry. Raman analysis is a technique which can obtain confirmatory class identification of analytes through low intensity laser light scattering. The technique is non-destructive, rapid, sensitive, and requires little or no sample preparation. Furthermore, portable Raman spectrometers are readily available, allowing for crime scene accessibility. Raman spectroscopy offers several advantages over the current methodology for GSR analysis. The technique has been shown to detect components from both the organic and inorganic constituents of GSR. This is contrary to current GSR elemental analysis methods which rely solely on the detection of the heavy metals (lead, barium, and antimony). This is problematic since environmental concerns have led to the increased popularity in heavy metal-free or "green" ammunition. It has been found that in the absence of heavy metals, current elemental analysis techniques are severely hindered when making accurate identification of GSR samples. Additionally, the probability of environmental and manufacturing particles assigned (incorrectly) as being GSR has increased with the onset of "green" ammunition. Until recently, the application of Raman spectroscopy for GSR analysis was largely unexplored, although this approach is not dependent upon detecting

metals, and is more capable of differentiating environmental contaminants and GSR. Therefore, a Raman spectroscopic method displays numerous advantages in specificity when compared to current techniques.

The firearm discharge process could be considered analogous to a complex chemical reaction. Therefore, the chemical composition of the products (GSR particles) is directly related to the chemical nature of the reagents (firearm-ammunition combination) and the conditions of the reaction. Preliminary results show that Raman data collected from GSR particles originating from different firearm-ammunition discharges were successfully classified according to caliber; 0.38 inch and 9mm caliber firearm discharge samples were probed using a 785-nm Raman excitation. Resulting data was treated with statistical methods (performed using Matlab with the PLS toolbox) such as Principle Component Analysis (PCA) and Support Vector Machines (SVM). The results show a high probability of this method to correctly classify data from the two examined calibers. Preliminary results illustrate that the variations between non-equivalent GSR samples can be detected through this method. Since GSR is often collected from a suspect, the application of this method to forensic investigations would provide a link between GSR collected from the shooter and the crime scene.

This emerging technique illustrates the possibility for an on-scene, non-destructive, identification and chemical characterization method for GSR. This method has the potential to greatly impact the forensic science community by increasing the accuracy (and discriminatory power) of GSR detection. The most direct application for this research is a method to exclude a specific firearm-ammunition combination as producing an evidentiary GSR sample. The comparison of a laboratory-generated GSR sample discharge and an evidentiary GSR sample, can be made without extensive preliminary studies.

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Gunshot Residue, Spectroscopy, Statistical Analysis

A109 Analysis of Lead-Free Ammunition by Scanning Electron Microscopy Using Energy Dispersive X-Ray Spectroscopy and Discrimination of Samples Using Multivariate Statistical Methods

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The goal of this presentation is to explore statistical methods for differentiating non-traditional Gunshot Residue (GSR) samples arising from six non-toxic, lead-free ammunitions. The formulation of non-toxic ammunition excludes the traditional signature composition of GSR, a mixture of lead (Pb), barium (Ba), and antimony (Sb). The removal of the unique combination of these three elements also renders differentiation of non-toxic GSR from environmental sources more challenging.

This presentation will impact the forensic science community by further exploring the combinatorial feasibility of Energy Dispersive X-ray Spectroscopy (EDS) and multivariate statistical analysis through the characterization of relatively understudied non-toxic ammunition. The application of these procedures allows for the statistical comparison of spectra, rather than the potentially more subjective visual comparisons. In turn, the statistical procedures allow comparisons to be made based on mathematical principles with measureable confidence in the comparison.

Traditionally, during the identification of GSR both morphological features of particles and elemental compositions are determined. In most laboratories, the presence of spherical particles containing Pb, Ba, and Sb indicates the definitive presence of GSR. However, the emergence of lead-free ammunition in response to health and environmental concerns requires a broader definition of particle compositions consistent with GSR. Unlike conventional primers, manufacturers of non-toxic ammunition may use vastly different elemental compositions to achieve the final product. For example, aluminum, silicon, potassium, strontium, and calcium may be included in the primer. Thus, no single particle composition can be easily amended to the description of GSR.

This study collected elemental profiles for six commonly available brands of non-toxic ammunition as well as for the most common brand of road safety flare utilized in the United States. Flares were included in the study due to their high strontium content and their potential for similarity in element composition to non-toxic ammunition. For each brand, one box of 9mm caliber ammunition was purchased and five rounds from each box were fired in the presence of three conductive carbon tabs positioned along the trajectory of the bullet, allowing capture of GSR. An additional five rounds from each box were disassembled to remove the propellant and bullet before being fired. These rounds were fired perpendicular to two conductive carbon tabs, allowing for the capture of only primer components. Road flares were burned in the presence of two conductive tabs positioned above the flare to capture any ejected particles during the combustion process.

A carbon tab was analyzed from each of the resulting 61 samples using a Scanning Electron Microscope (SEM). Particles visually consistent with the morphology of GSR were located on the tabs at an accelerating voltage of 20 keV. Once potential particles were discovered, the visual area of the sample was designated as a region of interest and all particles within view were analyzed by EDS. Searching of each sample finished after 30 regions of interest were identified. Each EDS spectrum was analyzed from 0-10 keV for elemental composition determination. Several different data pretreatment methods (including scaling and normalization procedures) were applied to the resulting spectra prior to statistical analysis. The pretreated data were then analyzed using Principal Components Analysis (PCA). This procedure is a useful visualization tool where samples with similar EDS spectra cluster closely in the resulting scores plot while samples with different spectra position further apart. In this study, PCA was first used to investigate association of ammunition by manufacturer, based on the element profiles generated. Then, the association of samples from whole bullets to those from primers only, and the differentiation of actual GSR from a ubiquitous road flare, were also investigated using PCA.

The application of data pretreatment and statistical procedures for SEM-EDS examinations of GSR allows greater confidence in results from a technique that is traditionally thought of as merely qualitative. Likewise, the lessons learned from the EDS analysis of GSR may also be applicable in the comparison of other items of trace evidence based on element composition, for example, comparisons of glass fragments or paint samples.

Gunshot Residue, SEM-EDS, Chemometrics

A110 Uncertainty Considerations for Measuring the Refractive Index of Glass

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After attending this presentation, attendees will understand the need for assessing uncertainty in glass refractive index measurements.

This presentation will impact the forensic science community by further validating the need to assess uncertainty in all areas of forensic science.

Refractive index has been used for comparison of glass analysis for many years. In this project, refractive index measurements were performed on 63 samples from 41 sources of glass using a glass reflective index system. Each sample was characterized by eight Refractive Index (RI) measurements collected at different places on the sample and some sources were characterized by up to three samples collected at different places on the source. Refractive index is

traditionally thought of as a means of classifying and differentiating glasses, but it is a numerical measurement and as such, should always be accompanied by an estimation of the uncertainty associated with the measured value. This is consistent with the recommendations made in the 2009 National Academy of Sciences Report. This project focused on determining a reasonable and defensible uncertainty estimate for refractive index measurements as taken in a typical forensic setting.

The results of this project reiterated the issue of inherent heterogeneity. This issue becomes more pronounced as the capabilities of the measurement process improve; the readability of RI measurements observed using double polarization light microscopy and refractive index oils may go to three decimal places while the glass reflective index system is capable of reliably determining five decimal places. There were several instances where samples taken from the source produced statistically significant different RI measurements. In casework, the ability to have many samples from a larger source may not be possible and, as a result, such differences could conceivably lead to an incorrect classification of a glass sample. A primary goal of this project was to establish the minimum number of RI measurements that should be taken across a sample to establish reasonable and defensible measurements of intra-sample variability.

Homogeniety of the glass sample is not the only factor of uncertainty in this study; other contributing factors including the calibration curve and instrument drift over time should be considered. In this project, these factors were incorporated into an uncertainty budget table. Here, a conservative approach was taken in which all contributing factors were kept in the budget; rounding at the end to the correct number of significant figures insured that factors were incorporated as their relative magnitudes dictated.

To evaluate the impact of retaining an uncertainty value for each RI measurement, pairwise comparisons of all 41 sources were analyzed to determine when the range of the two results overlapped. Here, the range around each RI was determined in two ways: first, from the standard deviation of the replicate measurements from each sample; and second, from the uncertainty budget, which led to the combined standard uncertainty value. This value was multiplied by a coverage factor of k=2, which roughly corresponds to the 95% confidence level. The number of pairwise comparisons more than tripled from 18 pairwise comparisons using one standard deviation to 74 pairwise comparisons using the expanded uncertainty. This clearly demonstrates the utility of using an uncertainty budget approach for RI measurements. In the event of casework and just the standard deviation was evaluated and used for RI determination, false positives or exclusions could have been made.

Statistical tests were performed on the three major categories of glass in this study; car windows, house windows, and bottles. Only car windows and house windows were found to have a statistically significant difference in their mean RI values; however, when the expanded uncertainty is considered, there is notable overlap between the three categories of glass. Therefore, the results of this study indicated that an RI measurement alone is insufficient to categorize glass based on source. Although this set of 41 sources does not comprise all of the possible sources that one could encounter in casework, the major categories of glass are present. This is also a smaller sample size compared to the larger picture, but the same types of trends are found here that are seen in the FBI study of float glass dating back to the 1960s.

Uncertainty, Refractive Index, Glass

A111 Forensic Glass Discrimination and Classification With Infrared Microprobe Analysis

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After attending this presentation, attendees will gain a better understanding of how infrared microprobe analysis can be applied to the forensic analysis of glass.

This presentation will impact the forensic science community by showing how infrared microspectroscopy can be used to aid in the discrimination and classification of glass evidence.

Glass is a ubiquitous material, and as a result, it is commonly recovered as transfer evidence when glass objects are broken during the commission of a crime. Vehicle windows, architectural windows, containers, headlamps, light bulbs, and mirror glasses are some of the major sources of this evidence at a number of crime scenes, including, but not limited to, car accidents, robberies, vandalism, bombings, and homicides. Broken glass is readily transferred to the breaker and to any individual or object in the vicinity of the breaking event; thus it has the ability to associate a suspect with a crime scene location or item and, in some cases, the time of occurrence. The significance of glass evidence is enhanced when the fragments are determined to be indistinguishable in all measured properties from the broken glass object. Conversely, if the recovered fragments differ in their measured properties from the broken glass object, then that glass object can be eliminated as a possible source of the glass from the suspect. The analysis of the molecular structure of glass is a novel forensic method that provides knowledge about glass chemistry that is not currently employed by forensic scientists as well as improves the discriminatory power within this class of transfer evidence.

Infrared (IR) spectra contain extensive information about the molecular structure of the complex silicates in commercial glasses. This research is based on measuring the Attenuated Total Reflection (ATR) mid-IR spectra of soda-lime silicate glasses to detect variations of the molecular structure to assist in the comparison of glass evidence.
The use of ATR mid-IR spectra for the discrimination and classification of glasses was investigated. Discrimination error rates of approximately 5% and classification by end-product (window or container) error rates of less than 2% were achieved with multivariate statistical methods, specifically Principal Component Analysis-Canonical Variate Analysis (PCA-CVA) and Partial Least Squares Discriminant Analysis (PLS-DA).

The mid-IR microprobe analysis of glass requires only minimal additional sample preparation to that which is already done for Refractive Index (RI) analysis and uses IR investigated samples that are the same size and smaller than the ablated hole made in Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS). Thus, mid-IR microprobe analysis can be used to provide additional discrimination for glass samples that are indistinguishable by RI and too small for elemental analysis. In addition, most forensic laboratories have IR spectrometers and/or microprobes which are used in the analysis of a plethora of evidence types including, but not limited to, paint, fibers, plastics, adhesives, and illicit drugs. Thus, this technique would not necessarily require a crime laboratory to purchase new instrumentation or require extensive training for trace evidence examiners. As a result, the use of ATR mid-IR microspectroscopy for forensic glass comparisons and analysis could potentially be implemented by forensic laboratories immediately and with minimal expense. ATR mid-IR spectral analysis provides information about the molecular structure of soda-lime silicate glass to support other traditional analysis and strengthen the association of this evidence.

Glass, IR Spectroscopy, Chemometrics

A112 Determination of Unique Compositional Patterns in Glasses by Micro-XRF: Multivariate Approach and Statistical Analysis

Sergey Mamedov, PhD, 3880 Park Ave, Edison, NJ 08820*

After attending this presentation, attendees will learn about application of micro-XRF to glass and soil analysis.

This presentation will impact the forensic science community by providing practical information about application of micro-XRF to chemical analysis.

X-ray Fluorescence (XRF) spectroscopy is a useful tool for identification of substances and confirming their identity with little or no sample preparation. New capabilities of the energy dispersive XRF analytical microscope (micro-XRF) enable the recording not only of spectra from small glass particles (as small as 50-100 microns) but also the hyper-spectral image of any object with high spatial resolution (<10 micrometers). Hyper-spectral image is a set of the data which contain information about position of the point along with full XRF spectrum at this point. This means that the data can be mined for unsuspected elements after the measurements have been made, and that statistical methods (multivariate analysis) can produce chemical distributions of the elements (Image analysis) and/or material classification based on Principal Component Analysis, in particular, with association between elements that can aid in identification of bonded phases. For example, statistical analysis of micro-XRF data for glasses can be used to locate the make, model, and year of car by analyzing a glass chip. This presentation will provide practical insights into the application of the micro-XRF to the analysis of glasses and soil.

An XRF analytical microscope was used in this study. This desktop unit utilizes a portable 50W X-ray source for excitation, two switchable (as small as 10 microns) monocapillaries for different spatial resolution, and the unique capability to work in vacuum (enhanced sensitivity for light elements), in partial vacuum, and under ambient conditions. In addition to the XRF spectrum/image, the XRF microscope provides a micro-transmission image of the material. Standard software package includes quantification using fundamental parameters method, basic statistical analysis for multi-point measurements, image analysis, and image processing.

X-ray fluorescence spectrum of the glass strongly depends on X-ray optical system, sensitivity of the detector, and accelerating voltage. In addition, background from the substrate will contribute to the spectrum of the small glass pieces because excitation X-ray penetrates through the glass and interacts with substrate. This effect becomes very important for particle size of 300 microns (or less) or powder. The change in the spectrum due to the shape or size will lead to the different quantification of the sample (different composition). A method which allows one to minimize this effect or take it into consideration was developed. In this presentation, examples of spectra from bulk material, small glass pieces, and powder will be shown.

Spectra of glass from several car manufacturers and commercial glasses (microscope slides, window glasses, fuse glass) in the range of 1.00-40.96 keV (<400 spectra) were collected and analyzed. Because only a few spectra have an additional features in the energy range above 15 keV, spectra were truncated and analysis was done in spectral range of 1.00-15 keV. Standard FPM algorithm without any correction and/or calibration was used to calculate concentration of Na_2O, MgO, Al_2O_3, SiO_2, K_2O, CaO, TiO_2, MnO_2, Fe_2O_3, As_2O_5, and CeO_2 in all samples. This set of concentration to build a data set for PCA was used. All spectra and concentration data sets were scaled before Principal Component Analysis was applied. Correlation between classification based on spectral analysis and concentration analysis will be shown.

Glass, XRF, Analysis

A113 Size Limitations for the Analyses of Float Glass Fragments by LA-ICP-MS: Influence of Fragment Size on Measurement Accuracy and Discrimination Potential for Glass Analysis

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After attending this presentation, attendees will understand principles concerning size limitations for the analyses of float glass fragments by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS), also addressing such issues as criteria to determine the minimum sample requirements, and effects of deviating from the standard protocol.

This presentation will impact the forensic science community by serving as a key aspect to raise awareness on the limitations of applying LA-ICP-MS in forensic glass analysis.

Over the last two decades, LA-ICP-MS has been proven to be a reliable and powerful technique in forensic glass analysis, especially for the comparative analyses of questioned and control glass samples. Harmonized measurement parameters have been established over time, leading to methods commonly used by the majority of forensic laboratories employing LA-ICP-MS. These methods are using single spot analyzes, multiple sampling positions on a single fragment, 10 Hz repetition rate, a crater diameter 50-80 micrometer, and an ablation time of 50 seconds or more. These methods commonly applied require a material amount for each analysis in the range of 500-1400ng assuming a crater depth of approximately 100 micrometer and crater diameter ranging from 50 to 80 micrometers. Applying six analyses on each glass fragment, the minimum sample requirement for a glass fragment to be suitable for analysis is approximately given by a minimum sample size of 0.4-0.5mm and a minimum thickness of 0.1mm.

In casework, a fair amount of recovered glass fragments might have a maximum-size length smaller than 0.5mm and many glasses exhibit a very small thickness (<0.1mm). Measurement of these fragments can be done with a reduced number of readings (ablation spots) or a shortened transient signal caused by thin fragments leading to depleted/reduced sample information. Based on the significantly smaller sample amount to be transported into the ICP-MS and on shorter integration intervals, a larger standard deviation that has an impact on the match criteria and a reduced discrimination power can be expected.

This presentation will evaluate the effect of smaller sample sizes on the analytical results (i.e., accuracy), and hence the discrimination potential of the method.

In this presentation, results from the investigation on over 70 fragments from three different float glasses will be presented. The selection of the glass samples was based on their different color and thermal history: a green non-tempered float glass (Vegla, Herzogenrath/ Germany), a clear non-tempered float glass (Flachglas, Weiherhammer 1/ Germany), and a green tempered float glass (Libbey Owens Ford, Lathrop CA/ U.S.A) were investigated. Glass fragments of different sizes were selected from each of these glasses and physical properties (size, mass) were documented. Samples were analyzed using a standard LA-ICP-MS method using helium as a transport gas.[1] The effect of the limited sample size on the analytical results and the impact of reduced analytical information on the discrimination power using a standard LA-ICP-MS method were investigated.

The evaluation was done applying pairwise comparisons of the data sets using a modified four sigma match criterion already described in literature.[2] Results of a particular fragment size were compared with the results from different fragments of the same glass and were also compared with the results of glasses of different origin in order to evaluate type one and type two errors. Also, the application of modified ablation parameters with reduced repetition rate and smaller spot size is described and evaluated.

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LA-ICP-MS, Float Glass, Sample Size

A114 Discrimination of Fiber Reinforced Plastics (FRP) Using Thermogravimetry (TG)

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After attending this presentation, attendees will understand how the analyses of samples concerning collision cases between ships are performed in Japan. In addition, this presentation will introduce the method for identification of Fiber/Glass Reinforced Plastics (FRP/or GRP) using Thermogravimetry (TG).

This presentation will impact the forensic community by demonstrating a method to discriminate between thermoset polymers not only for ships collision cases but also for land criminal cases since TG has not often been used by the forensic community.

In Japan, there are on the average 30 cases a year of hit-and-run collision accidents between ships, as well as some accidents where the crew on duty does not recognize the collision. For investigation of these cases, paints which are transferred from the hull of one ship to another would be the most valuable type of evidence. Paint samples taken from both ships are observed to determine if they have the same appearance (sequence of layers, colors, etc.). Each layer is then analyzed and compared using Fourier-transform infrared microscopy and scanning electron microscope-energy dispersive X-ray spectroscopy.

Smaller vessels such as fishing and pleasure vessels are commonly built of Fiber Reinforced Plastics (FRP) and are surfaced with a “gel coat” often colored white. When FRP ships collide with other ships, pieces of gel coat (sometimes with paints) are likely to be transferred and collected as samples. The gel coat is an unsaturated polyester (UP) resin-based plastic matrix with some pigments and additives. There are traditionally two major types of UP resin used for vessel construction; orthophthalic (ortho); and isophthalic (iso). The major pigment is always TiO_2 because of its color and there are also other pigments/fillers (clay, talc, etc.) included in small amounts. When ships get older, they sometimes have single or multi-layered paint, but newly built FRP ships have no paints on the gel coat (except for antifouling paint on the bottom of the hull). Therefore, it has been recognized to be difficult to characterize gel coats. This presentation will examine the data of gel coat samples which were analyzed in 28 collision cases since 2007. Among the samples from 28 cases, 14 were ortho (10 had paint layer(s) on their surfaces), 13 were iso (four painted), and one was an other type of polymer resin (not painted). Although all 14 ortho samples, six of the iso samples, and the one resin were discriminated by their own IR spectra or by variety of their paint layers, it was difficult to distinguish two pairs and a group (containing four samples) of iso’s without paint on their surface. In order to characterize these gel coats, a further analysis method was required.

TG is a popular method for evaluating polymer properties; however, the use of this technique for forensic purpose has rarely been reported. In this presentation, the result of the TG of gel coats will be introduced. The primary goal of this study was to optimize the experimental conditions; atmosphere, temperature range, heating rate, and sample amount. Several gel coat samples collected from dockyards were analyzed with thermogravimetric differential thermal analyzer (TG-DTA). All of the thermogravimetric curves showed at least three degradation processes under air atmosphere. By comparing the temperatures corresponding to the maximum rate degradations, some pairs of samples which have no differences in IR spectra and elemental analysis had distinct discrepancies. The differences between samples may be attributed to the minor additives and cross-linking structure of polymers.

FRP, TG, Paint

A115 Systematic *In Situ* Identification of Pigments in Paint by Raman Microspectroscopy

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The goal of this presentation is to demonstrate ways in which Raman spectroscopy can be incorporated in a forensic laboratory to improve the evidentiary significance of paint evidence.

This presentation will impact the forensic science community by suggesting the first systematic organization of pigment analysis that is broadly applicable to forensic paint analysis.

Colorants (pigments and dyes) surround us in everyday life, yet beyond spectrophotometric comparisons of bulk color, forensic analyses typically ignore the colorants within a sample. This is due in large part to the fact that colorants are present at low concentrations, and pigments are, in addition, typically quite small (<1µm), making them difficult to access analytically. Successes have been achieved through a number of microanalytical methods, including polarized light microscopy, infrared microspectroscopy, microchemistry, micro x-ray fluorescence, and X-ray diffraction; however, the application of each of these approaches is limited by one or more factors such as analytical volume, specificity, sample preparation requirements, or the level of interpretation expertise required.

Raman microspectroscopy suffers from two of its own major limitations, which are fluorescence and the current lack of a systematic approach to pigment identification. Fluorescence can be minimized through the use of multiple laser wavelengths, photo bleaching, and background removal, yet it remains the Achilles heel of the technique, leaving some pigment spectra inaccessible by this method. The second issue, regarding interpretation, is being reduced by the fact that more and more laboratories are obtaining and using Raman systems; however, the framework for analyzing and interpreting paint evidence remains limited. This presentation, whose primary focus is to address this shortcoming, will provide an overview of our past six years of research, which has focused on the development of: (1) a pigment identification scheme; (2) laboratory methods designed to optimize pigment analysis; and, (3) of interpretation and significance studies that will provide a framework for assessing the value and uses of pigment information.

Through the development of an extensive database of pigments (>1,100 pigments and >325 unique pigments), a classification scheme for the systematic identification of pigments was devised. Each pigment in this database has been carefully verified by orthogonal methods and categorized by chemical structure. The Raman spectra and pigment chemistry have been used together to develop a flowchart that permits classification of pigments on the basis of strong peaks, which is intended to be applicable at the bench level. Within the context of an investigation, it also permits an examiner to determine which pigments can (and can’t) be discriminated on the basis of strong peaks in a sample spectrum. This organization provides the first systematic basis for addressing pigment identification in forensic-sized samples. It also shows that of the 325 unique pigments studied, only 38 (~12%) had strong enough fluorescence that no useful Raman scattering was observed (using 514 and 785nm lasers).

Building upon this identification scheme, the second phase of this research focused on the *in situ* identification of pigments in paint, with a goal of systematically addressing the evidentiary significance of pigment identification within a polymer matrix. Various methods of sample preparation and interpretation were evaluated to determine whether any methods are optimal. The analysis focused on a collection of 300+ late model automobile paint samples (comprising over 1,000 paint layers) and several sets of architectural tinting pigments (used to color the majority of architectural paints in the U.S.) collected over recent years.

The color layers in all of these paints (over 500 layers), which included top coats, tinted clear coats, and coordinated primers, as thin as 5µm in cross section, were analyzed by micro-Raman spectroscopy to identify the pigments present. In total, 28 different pigment groups were identified among the automotive paints and 14 pigments were identified within the architectural tinting colors. Paint pairs from the automotive paint collection of the same color code were also characterized by micro-FTIR spectroscopy. These results were interpreted to evaluate the potential applications of Raman spectroscopy in a trace evidence lab, with a specific focus on evaluation of the method as: (1) a fast screening method (for with no preparation); (2) to provide additional discrimination in conjunction with existing micro-analytical methods; and, (3) as a method to provide manufacturer sourcing assistance based on the identification of specific pigments. Each of these applications will be expanded upon in the presentation.

Paint, Pigments, Raman Spectroscopy

A116 Differentiation of Yellow Polyester Fibers With Different Dye Loadings Using Microspectrophotometry and Chemometrics

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After attending this presentation, attendees will gain a fundamental understanding of the application of multivariate statistics to the analysis of fibers.

This presentation will impact the forensic science community by touching upon key improvements suggested by the 2009 National Academy of Sciences Report. In particular, human observer error is a potential problem in fiber color comparisons and the use of multivariate statistics could virtually eliminate this issue.

Microspectrophotometry (MSP) is a quick, accurate, and reproducible way to compare colored fibers for forensic purposes. In turn, the use of chemometric techniques applied to MSP data can provide valuable information, especially when looking at a large dataset. As of now, routine fiber color comparisons are made by use of an MSP and the trained eye of a forensic fiber examiner, who examines the overall shape of a fiber's spectrum. Comparisons are made by overlaying the spectra from items of questioned and known origin and determining if similar spectral characteristics are observed. However, minute differences not seen by the examiner can provide valuable information in a relatively featureless spectrum. The use of chemometric techniques like agglomerative hierarchical clustering (AHC), principal component analysis (PCA), and discriminant analysis (DA) can detect these differences and make objective comparisons of complex data possible.

The purpose of this study was to use chemometric techniques to discriminate UV-visible spectra obtained from yellow polyester fibers that had different dye loadings. Research has shown that visually similar yellow polyester fibers can be discriminated based on their UV-visible spectra; however, none have determined if fibers dyed with the same dye, but with different dye loadings, can be discriminated by their UV-visible spectra alone. Background subtracted and normalized UV-visible spectra from 11 yellow polyester exemplars dyed with different concentrations of the same dye ranging from 0.1-3.5% were analyzed by AHC, PCA, and DA. Simple visualization of the overlaid spectra showed the shape of the spectral curve broadened as dye loadings increased. One fiber with an unknown dye loading was determined to have a dye loading between 0.75-1.5% based on visualization of the spectra and subsequent chemometric techniques. AHC and PCA grouped the fibers into three classes ranging from low to high dye loadings. When grouping the fibers into their three classes based on low, medium, and high dye loadings, the classification accuracy was 94%. However, when fibers were grouped individually, the classification accuracy was quite poor (52%). In addition, an external validation study resulted in higher classification accuracy when fibers were grouped into three classes instead of individual groups (95% vs. 50%). Finally, exemplars with similar dye loadings were treated like known and questioned fibers and analyzed by PCA and DA in order to determine if they could be discriminated. Three exemplar comparisons and both class comparisons were considered discriminated based on a classification accuracy of 90% or higher and a receiver operating characteristics curve score of 0.9 or higher.

Overall, chemometric analysis of UV-visible spectra provides an objective means of discriminating similar fibers with different dye loadings.

Chemometrics, Dye Loadings, Fiber Comparison

A117 Examination of Statistical Methods for Analysis of Highly Similar Absorbance Spectra From Textile Fibers

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The goal of this presentation is to report on the evaluation of statistical methods for comparison of textile fibers with highly similar UV-visible absorption profiles.

This presentation will impact the forensic science community by providing information on the use of standard statistical techniques for performing comparison of highly similar microspectrophotometry data from textile fibers.

Discriminating clearly dissimilar absorbance spectra from questioned and known sources is straightforward; however, analyzing two fibers with nearly identical absorbance profiles can prove challenging. The goal of this research is to apply established statistical tests to highly similar absorbance spectra with the intent of evaluating the spectral match at a defined significance level. The nonparametric permutation test, Hotelling's T^2 test and Student's t-test were examined. The Parametric tests were applied to reduced dimensionality data preprocessed by principal components analysis (PCA).

An outside company was contracted to dye two 10 gram swatches of spun nylon 6.6 fabric. One swatch was vat dyed with Acid Blue 25 (M1); the other with Acid Blue 41 (M2). Absorption measurements were collected over the range of 400-725 nm with a microspectrophotometer. Samples of M1 were taken from three different areas (A1, A3, and A5), corresponding to the top left corner, center, and bottom right corner, respectively. Three individual threads (T1 – T3) were cut from both the vertical (D1) and horizontal (D2) directions of M1 in each of the three areas for a total of eighteen threads. After cutting all threads to 1cm lengths, five individual fibers were pulled from each thread (F1 – F5). Fifteen measurements were collected along the length of a single fiber. The spectra along each fiber were collected sequentially, while the order in which each fiber was examined was determined by random numbers drawn from a uniform distribution.

Pairwise comparisons of all fibers from M1 were performed by the nonparametric permutation hypothesis test, returning 100 p-values for each pairwise comparison. An average of 87% of all pairwise comparisons resulted in discriminations at α=0.05. This approach is not dependent upon a normal distribution of the comparative figures of merit. In parametric tests, any deviation from the assumed distribution may shift the actual size of the Type I error; however, in contrast, the actual α level of the permutation test automatically holds because when the null is true, all test statistics are exchangeable, having the identical distribution.[1] The high percent discrimination of fibers from M1 raises questions regarding the sensitivity of the statistical test, given the intended purpose, and possible sources of uncontrolled error in the experiments.

The nonparametric permutation method results were compared with those from the parametric Hotelling's T^2 test and Student's t-test. PCA was performed on normalized spectra from A5 of M1 to reduce the dimensionality of the data. Typically, greater than 99% of the variance was contained in the first principal component (PC1) for all comparisons. The large fraction of the variance contained in PC1 demonstrates the high spectral similarity. Student's t-test performed on the scores from PC1 allowed accepting the null hypothesis (i.e., the spectra were not discriminated by the test) in all of the five pairwise comparisons at $\alpha = 0.05$. Results from the Hotelling's T^2 test on scores from PC1 and PC2 accepted the null hypothesis in two out of five (40%) pairwise comparisons.

To further test the methods, samples from different materials M1 and M2 were analyzed. PCA of highly similar spectra from the two sources contained 98.6% of the variance in PC1 and 99.92% of the variance in PC1 and PC2. The Student's t-test on scores from PC1 accepted the null hypothesis at $\alpha=0.05$ (i.e., two fibers containing different dyes with highly similar spectra were not differentiated), while the Hotelling's T^2 test on scores from both PC1 and PC2 rejected the null hypothesis at the same significance level.

Further studies are focused on defining a statistical approach that can be applied to casework samples while maintaining Type I and Type II errors at reasonable levels.

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Spectroscopy, Fiber Analysis, Hypothesis Testing

A118 A Statistical Approach to Discrimination and Match Capability to Provide Scientific Basis for Estimating Significance of Fiber Association in Forensic Practice

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After attending this presentation, attendees will be introduced to the statistical significance of measurement variance of like and unlike fibers and will learn how the estimations of match significance can be made from fiber evidence. This is conducted through the use of multivariate statistical analysis and the product rule of independent variables.

This presentation will impact the forensic science community by demonstrating sources of variability and decision-making processes gained from this research that will serve to advance the forensic significance of class evidence involving fiber examinations.

The presentation will show how to increase the ability to classify and discriminate synthetic and cotton fibers. A library of over 800 well characterized fibers was developed with known dye components. Using visual light microscopy, visible Microspectrophotometry (MSP), and Fourier transform infrared spectroscopy, data was collected on fibers. In this report, studies were done on twenty-one red cotton and twenty-one red acrylic fibers using multivariate analysis. The absorption spectra of fibers from 10 replicate visible microspectrophotometry scans on each fiber were compared by using Principal Component (PCA) and Linear Discriminant Analysis (LDA). The software used in this work was developed at the University of South Carolina. PCA is used to find the directions of maximum variability to reduce the data dimensionality and enable use of LDA to provide projection maps of the data providing best discrimination of fiber groups. After projection into a two- or three-dimensional discriminant maps, discrimination of fiber groups can be judged visually by drawing 95% confidence limit ellipses around each group of points representing replicate spectra from the same fiber. Additional statistical hypothesis testing with Hotelling's T^2 test for the equality of means can be employed as a match criterion. With the aid of multivariate statistics, fibers that are difficult to distinguish by visual comparison can be distinguished. All 21 red cotton fibers, with the exception of two fibers, were distinguished by either PCA or LDA. The two that were not totally separated had the same dyes, but one of the fibers had a finish on its surface. PCA and LDA were also used to compare like fibers to determine how well fiber color matched on similarly dyed fibers. Separate samplings of the same red fiber measured seven weeks and two days apart showed large overlap and high correlation coefficients, thus providing quantitative match criteria. PCA and LDA provided a statistical basis to show the variance of like sample measurements and the discriminating capability of similar but different data. The next step required is to determine the probability of two statistically matched materials, based on measurements, as having come from the same source.

The "gold standard" in forensic science is the approach used in DNA matching by calculating the probability of occurrence of a given combination of alleles in short tandem repeats by the product rule of probability. By knowing the number of fibers in the database with specific color, diameter, cross-sectional shape, and chemical composition, the occurrence percentage of each fiber was determined. The product of the percentages was then calculated to determine the probability of two fibers matching randomly with those characteristics. Probabilities on the order of one in 0.5 million were obtained with such comparisons between fibers, and are valid, provided a sufficiently large and representative database of fiber characteristics is accessible. The improved understanding of sources of variability and decision-making processes gained from this research will serve to advance the forensic significance of class evidence involving fiber examinations.

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Fibers, Multivariate Analysis, Statistical Analysis

A119 On the Random Presence and Discrimination of Purple Textile Fibers Collected From Movie Theater Seats

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After attending this presentation, attendees will realize how unlikely it is to randomly observe a large number of matching fibers within a given surface and also between different surfaces.

This presentation will impact the forensic science community by providing information, not only related to the ability of microscopical examinations to detect and discriminate microscopic features of textile fibers, but also about the value of the recovery of a large number of matching fibers. This study will benefit trace evidence examiners in cases where a defense strategy would raise an argument such as "the recovered fibers are common and ubiquitous; therefore, this recovery is valueless to this case."

Adhesive tapes have been applied to 150 seats of a movie theater. These public surfaces were targeted for fiber collection because they were considered to be representative of the general population where the study was conducted. The focus of this study was purple fibers: this color was chosen to deviate from the traditional colors like black, blue, or red which are already abundantly reported in the literature. Single purple fibers were searched for using a stereomicroscope and isolated from the tape. To date, 20 seats were searched for fibers: 7,000-8,000

fibers were present on the searched tapes. A total of 62 purple fibers were observed. The single fibers were mounted permanently for conducting microscopical examinations. Methods based on light microscopy are crucial for gathering data about fiber characterization as well as for performing comparisons. The study of the morphology of the fibers is first carried out; this may include the study of the cross-sectional shape. Fibers can thus be classified as natural or man-made. Their thickness can be measured, the presence of delusterant particles can be recorded for man-made fibers, and double polarization techniques are applied to study their optical properties. Fluorescence microscopy is useful to detect luminescence due to the dye content. Plane-polarized light can also be used to study potential dichroic properties of the dyes. The comparison microscope is used to perform intra-source and inter-sources comparisons: the color, thickness, presence and concentration of delusterant particles, surface appearance, and fluorescence properties are compared.

During this study, fibers were initially categorized according to their general class: about 50% of the collected fibers were natural, the most part being cotton. Few wool fibers were observed. About 25% of the fiber samples were of man-made regenerated origin and the other 25% were man-made synthetic types (the most part being nylons and polyesters). The various fiber samples belonging to a given subclass were pair-wise compared using the comparison microscope. The results indicate that this population of purple fibers is highly variable, especially considering the different color shades, their thickness, and their overall morphology. Color resulted to be the highest factor of discrimination. Indeed, a further step of this study will be the application of microspectrophotometry.

After comparison microscopy, a group of eight cotton fibers resulted to be indistinguishable and this was the largest group. Four fibers were recovered from the same seat, while the remaining four were collected from four individual seats. The group of four was confirmed after fluorescence microscopy along with another fiber of the group of eight, while the three other fibers exhibited different fluorescence effects. For cotton fibers, two groups of three indistinguishable fibers were observed: one group had two matching fibers recovered from the same seat. For the other group, the three fibers were collected from three different seats. On the other hand, all the regenerated fibers could be differentiated after comparison microscopy. With regard to the synthetic fibers, three pairs were observed. However, the most useful way to gather information about the recovery of these fibers is to consider the number of groups of purple fibers recovered on a given seat and their size. Most of the purple fibers occurred as individual fibers on the seats. Only one seat had more than two groups of purple fibers (two pairs of cottons and synthetic fibers, respectively). Globally, seven pairs of matching fibers were observed on six seats. Finally, the largest group observed from one seat was only four fibers.

Fibers, Evidential Value, Trace Evidence

A120 Analysis of Allelic Drop-Out Using the Identifiler® and PowerPlex® 16 Forensic STR Typing Systems I: Estimation of Drop-Out Probabilities

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After attending this presentation, attendees will learn how empirically derived drop-out probabilities can be obtained using low-template DNA profiles that were run as part of their laboratory's internal validation studies, and how these probabilities perform well when used on model evidentiary samples. They will then discover how the calculation of dropout probabilities can be incorporated into a likelihood ratio that assesses the strength of the DNA evidence under competing hypotheses.

This presentation will impact the forensic science community by providing a means of assessing the weight of low-template DNA evidence that requires consideration of allelic drop-out, also providing a means to increase accuracy and objectivity in interpreting DNA profiles.

Low-template (LT) DNA profiles continue to present interpretational challenges to the forensic community. Whether the LT contribution comprises the main profile, or whether it is present as the minor component of a mixture, ambiguity arises from the possibility that alleles present in the biological sample may not be detected in the resulting DNA profile. This phenomenon is known as allelic drop-out. This ambiguity complicates both the assessment of the potential number of contributors as well as an estimation of the weight of the DNA evidence for or against specific propositions. One possible solution to estimating the weight of the evidence is to use a likelihood ratio (LR) that incorporates the probability of allelic drop-out $P(D_O)$. Such methods can be improved by including an estimate of the drop-out probability for the specific evidence sample. However, while a vast repository of data exists from which dropout probabilities might be calculated, few empirical studies to determine such probabilities have been performed to date. Here patterns of allelic drop-out are characterized using the Identifiler® and PowerPlex® forensic STR multiplexes in single-source samples generated by the National Institute of Standards and Technology (NIST). Briefly, DNA was adjusted to amplify 100pg, 30pg, or 10pg from each of two individuals. Each of the six samples (three concentrations of each of the two individuals) was amplified 10 times with both the Identifiler® and PowerPlex® 16 kits using the standard number of PCR cycles suggested by the manufacturer. Thus, a total of 60 profiles were produced for each kit.

Crucial to the determination of dropout probabilities is the selection of an appropriate detection threshold. A threshold derived from analytical chemistry, designed to properly balance signal and noise, was used to evaluate the samples, as well as thresholds commonly used in forensic DNA laboratories. The effect of these different allelic detection thresholds on observed patterns of drop-out were then evaluated. Drop-out was defined to be the situation where a particular peak known to exist in the sample does not rise above the allelic detection threshold, and, as a result, is not detected in the profile. Not surprisingly, fewer instances of apparent drop-out were found when using a lower detection threshold.

Logistic regression to model the fraction of alleles that dropped out of a profile as a function of the average height of the detected peaks was used. The equation derived from the logistic regression model allowed the authors to estimate the expected drop-out probability for a model evidentiary sample based on the average peak height of the profile. The correlation of the proportion of allele drop-out from a profile with the average peak heights within a particular profile supports using logistic regression to model the relationship between these two variables. In several cases, the parameters of the logistic regression models differed significantly between typing systems, different allelic detection thresholds, and samples. Finally, a positive correlation exists between allele drop-out and allele length; longer alleles tend to drop-out more frequently than shorter alleles, even in good quality samples. These results provide an initial foundation for empirically estimating drop-out probabilities that can be incorporated into LR calculations to assess the weight of complex DNA evidence including LT components.

Dropout Probabilties, Logistic Regression, Likelihood Ratios

A121 Analysis of Allelic Drop-Out Using The Identifiler® And PowerPlex® Forensic STR Typing Systems II: Evaluation of Estimated Drop-Out Probabilities

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After attending this presentation, attendees will appreciate how empirically based drop-out probabilities can be obtained for low-

template DNA profiles. Further, they will learn how a simulation approach can be used to determine the accuracy of the drop-out probabilities.

This presentation will impact the forensic science community by objectively exploring the performance of likelihood ratio approaches to assess the weight of low-template DNA evidence that require consideration of the allelic drop-out. The work presented provides a means to increase the accuracy and objectivity in interpreting DNA profiles.

One major challenge in the analysis of forensic DNA profiles concerns the analysis of low-template (LT) DNA samples. Due to the small number of DNA molecules analyzed, such samples may not yield the complete profile(s) of the contributor(s). It is difficult to correctly interpret such profiles because some of the information may be missing. Further, it is even more challenging to accurately assess the weight of such evidence.

To fill this void, patterns of allelic drop-out were recently characterized using the Identifiler® and the PowerPlex® forensic STR multiplexes in 60 LT single-source samples. Logistic regression was employed to model the relationship between the fraction of alleles that have dropped out of a profile as a function of the average height of the detected peaks. This model allowed the estimatation of the drop-out probability for a simulated low-template LT evidentiary sample based on the average peak height of the profile. These estimated drop-out probabilities could then be incorporated into a likelihood ratio (LR) to assess the strength of the LT DNA evidence.

However, one concern is that there were several statistically significant differences in the relationship between peak heights and drop-out between different samples and experimental conditions. The practical effect of these differences remained unclear. Thus, previous work was expanded and extensive simulation studies were performed to evaluate the accuracy and practical applicability of estimated drop-out probabilities.

Each of the LT DNA profiles, simulating an evidentiary profile, was compared with the profile of a suspected contributor. For each of these comparisons, two different LRs were computed. The first one used the estimated drop-out probability. The second LR used the true drop-out probability. If the estimated drop-out probabilities are accurate, then the LRs calculated using them should be similar to those calculated using the true drop-out probability.

Two different types of comparisons between LT profiles and suspected contributors to assess how well the drop-out estimator functions were analyzed. The first type of comparison evaluated the true contributor as the suspected contributor of the LT DNA evidence. The second type of comparison evaluated a true non-contributor as the suspected contributor of the LT DNA evidence. Here, the suspected contributor was a random individual simulated from a U.S. population database with European ancestry. For all comparisons, LRs were calculated using the Balding and Buckleton (2009) R program.

For both types of simulations, the LRs calculated using the estimated drop-out probabilities were similar to those calculated using the true drop-out probabilities, suggesting that the estimates of the drop-out probability are accurate and useful. This trend holds even when using the data from the PowerPlex® 16 typing system to estimate the drop-out probability for an Identifiler® profile and vice versa. Thus, even though some of the logistic regression model parameters differ significantly from each other across different experimental conditions, they are similar enough to have little practical effect on the final LRs calculated using these estimated drop-out probabilities. This research demonstrates that use of an LR that incorporates empirically estimated allelic drop-out probabilities provides a reliable means for extracting maximum information from LT forensic DNA profiles.

Low Template DNA, Drop-Out, Likelihood Ratio

A122 Effects of Relatedness on Likelihood Ratio Calculations for DNA Mixtures

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After attending this presentation, attendees will understand how relatedness among contributors to forensic DNA mixtures can affect likelihood ratio calculations when unknown contributors are modeled as unrelated, also understanding the related contributors as known in the model can nullify some of these effects.

This presentation will impact the forensic science community by exposing some of the strengths and limitations of likelihood ratios for forensic mixture analysis. Most laboratories wrestle with complex mixture analysis and relatedness among contributors and suspects can further create additional complications.

A likelihood ratio (LR) can be used to assign a statistical weight to a comparison between a DNA mixture obtained from an item of evidence and the DNA profile of a known individual, such as a suspect. An LR is a ratio of two probabilities. In forensic DNA analysis, these are typically the probability of the DNA mixture conditional on a prosecution hypothesis (H_p), often a scenario that includes a suspect, and the probability of the mixture conditional on a defense hypothesis (H_d), where the suspect is replaced by an unknown person. There may be one or more additional unknown persons in either or both scenarios. These unknown persons are most often assumed to be unrelated to one another and to the suspect.

If contributors to a mixture are related to one another or to the suspect and this is not considered in the formulation of the LR, the LR may be conservative or anti-conservative. As a consequence of relatedness, a mixture may appear to be composed of fewer than its true number of contributors, due to allele sharing. If this happens, the scenarios selected for the LR may be incorrect. As a consequence of relatedness between mixture contributor(s) and a non-contributing suspect, the suspect's alleles may appear in the mixture by chance. To explore these two possibilities and their effects on the LR, DNA profiles of related and unrelated individuals were simulated and theoretical mixtures were created.

Suspect profiles and six different types of three-person mixtures were simulated such that the suspect was not a contributor, but one or more of the suspect's relatives were contributors. Mixtures included: (1) two of the suspect's siblings and an unrelated person; (2) one of the suspect's siblings and two unrelated persons; (3) two of the suspect's half-siblings and an unrelated person; (4) One of the suspect's half-siblings and two unrelated persons; (5) The suspect's sibling, parent and an unrelated person; and, (6) two of the suspect's cousins and an unrelated person. Mixtures were also simulated including the suspect as a contributor and without allelic drop-out and drop-in.

Likelihood ratios were computed using the Forensic Statistical Tool (FST), developed and used for mixture analysis in casework by The Office of Chief Medical Examiner (OCME) of the City of New York. FST employs empirically determined drop-out and drop-in rates, so the contributors in the prosecution and defense scenarios need not fully explain the alleles in the mixture. For each mixture, H_p and H_d were formulated with and without the suspect's relatives and the unrelated persons as known contributors to the mixtures. After testing with the suspect, LRs were also computed for the same scenarios using a set of other non-contributors as the "suspect." This demonstrated the range of LRs expected for unrelated non-contributors and was used to determine whether the related suspect's LR was artificially inflated.

Analysis of the simulated mixtures showed that a non-contributing suspect's LR can sometimes be inflated when one or more of the suspect's relatives contributed to the mixture, particularly when the mixture included two of the suspect's siblings. However, this inflation can be mediated by inclusion of the true contributors as knowns in the LR. Therefore, it is important to attempt to obtain elimination samples from individuals related to the suspect if they may be part of the mixture. If it is not possible to obtain the elimination samples, the LR may be computed without known contributors, but it should be emphasized that

the current model treats unknown contributors as unrelated. Ultimately, it may be necessary to develop LR calculation software that can account for relatedness through appropriate algorithms.

Likelihood Ratio, DNA Mixtures, Relatedness

A123 How Inclusion Interpretation of DNA Mixture Evidence Reduces Identification Information

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After attending this presentation, attendees will better understand how human review of DNA mixture evidence using the inclusion method reduces identification information.

This presentation will impact the forensic science community by enabling practitioners to preserve more identification information in their DNA evidence, reaching the conclusion that human interpretation may discard considerable DNA mixture identification information that computers can preserve, which can improve criminal justice processes and outcomes.

The combined probability of inclusion (CPI) mixture interpretation method applies thresholds to quantitative STR peak data in order to simplify human review. However, this flattening of peaks into putative all-or-none allele events usually reduces evidential force. The true DNA match statistic is better estimated by a computer model that accounts for peak heights and their variation.[1]

The "weight of evidence" is an additive measure of DNA identification information, calculated as the logarithm of the likelihood ratio (LR) and measured in "ban" information units. Since CPI can be viewed as an LR, this study looked at log(LR) at individual STR loci to determine how CPI reduces match information.

The study examined 615 locus experiments obtained from 41 inclusions in 31 mixture evidence items that had a reported CPI statistic. The items were from 16 cases, and included clothing, weapons, vehicles, vaginal swabs, and skin swabs. Promega PowerPlex® 16 STR electropherograms were developed on an ABI 3130® genetic analyzer, and reinterpreted on Cybergenetics TrueAllele® genetic calculator.

There were 517 loci (84.1% of 615 experiments) for which both CPI and computer match statistics were calculated. At these loci, the computer found an average log(LR) of 0.746 ban per locus, forming a bell-shaped normal distribution having standard deviation 0.590. DNA evidence need not support an identification hypothesis at every locus, as was seen in the 52 (10.1%) experiments having negative log(LR) values.

On these 517 loci, CPI yielded an average log(LR) of 0.489 ban per locus. CPI only reports on loci that support an identification, and is silent about evidence whose weight does not support an inclusion. The CPI weight of evidence values formed a truncated normal distribution. These positive log(LR) numbers had a maximum at 0 ban per locus, monotonically decreased to the right, with a fitted standard deviation of 0.615. Comparison with the better-modeled computer log(LR) distribution showed that: (1) on average, CPI reduces identification information relative to a computer gold standard; (2) CPI discards locus evidence that does not support an inclusion; and, (3) CPI can report an inclusion at a locus where the computer finds no support for a match.

When using CPI, a locus is not reported unless evidence alleles over threshold are seen in the reference genotype. There were 97 loci (15.8% of 615 experiments) at which the computer found a statistic but CPI did not. At the 68 (70.1%) of these unreported CPI loci that had interpretable data, the computer produced log(LR) values in a normal distribution having a mean of 0.659 ban per locus and standard deviation 0.664. At the remaining 29 (29.9%), experiments exhibiting allele dropout or extreme peak imbalance, the computers log(LR) values were all negative, having mean –0.755 ban per locus and standard deviation 0.346. Overall, computer interpretation of loci that CPI did not use contributed additional weight of evidence that favored an identification.

In this study, reported CPI locus statistics with more sophisticated computer reinterpretation of the same mixture data were compared. Whenever CPI produced a result, so did the computer. However, CPI yielded (on average) a lower weight of evidence than the computer; CPI discarded evidence whenever it classified a locus as unfavorable; and CPI reported as favorable some loci that the computer found to be unfavorable. For more accurate and balanced reporting of DNA mixture evidence, laboratories using CPI should progress to more informative interpretation methods.

Reference:

1. Perlin MW, Legler MM, Spencer CE, Smith JL, Allan WP, Belrose JL, Duceman BW. Validating TrueAllele® DNA mixture interpretation. J Forensic Sci 2011;56(6):1430-47.

Interpret Mixture, Inclusion Method, Weight of Evidence

A124 SNPs, Forensic DNA Mixtures, and Population Genetics

Kevin C. McElfresh, PhD, 13580 Groupe Dr., Ste 301, Woodbridge, VA 22192*

After attending this presentation, attendees will have a better understanding of analyzing forensic DNA samples containing mixtures of DNAs.

This presentation will impact the forensic science community by providing advanced analytical capabilities for DNA mixtures that cannot be analyzed using STRs.

The essence of forensic DNA analysis is the comparison of known differences in the human DNA of one sample with the differences of an unknown sample. Match or non-match is based on the sum of the differences between the two samples. If a difference is characterized in binary code of one for complete difference and 0 for no difference, then in a comparison of single source forensic DNA samples, if that sum is zero, the samples match, but if that sum is non-zero the samples do not. The problem lies in the comparison of mixtures of DNAs. In this case, there is the distinct possibility that there is an inclusion hidden beneath the non-zero answer. In the case of STRs, mixtures beyond two individuals virtually negate the ability to attempt an analysis of the zero answer within the non-zero aggregate. Ultra-High Density Single Nucleotide Polymorphism (UHD-SNP) arrays analyzing >5 million loci across the human genome provide an alternative forensic DNA mixture analysis capability due to the smaller number of alleles per locus that are compensated for by the larger number of loci. Thus, the smaller numbers of alleles (two) at an SNP locus allows for the assessment and aggregation of small per locus differences over a large data set and determine the effect of the zero-based inclusion within a non-zero aggregate. The objective of this project was to determine the fundamental effects of reference population and number of SNP loci necessary to correctly assess the contributors to a forensic DNA mixture.

Computer simulations of SNP data done by Homer and Jacobs, using different methods of calculation, both addressed the possible analytical capabilities for mixtures of DNAs within a medical and forensic context (Homer) and in a medical Genome Wide Association context (Jacobs).[1,2] In the computer simulations, the percent contribution to the mixture was simulated and tested using prepared mixtures. In addition to the mixture proportion, there was a clear component of reference population to the analysis. This study has examined those calculations using samples from forensic mixtures utilizing samples from typical forensic crime scenes, e.g., blood/semen mixtures and also complex mixtures of more than two individuals.

A baseline of fundamental characteristics of SNP results was developed using single source match and non-match DNAs taken from blood and semen. These included serial dilution of DNAs and subsequent comparison of the number of loci and the effect of the population analysis on the results. From these studies a clear baseline of Loss of Detection (LOD) and sample analysis criteria were drawn.

Reference populations were built for Caucasian, African, Hispanic and Asian populations. The population specific results were then compared using the Illumina provided SNP cluster file and population specific cluster files using these criteria. The same analysis was then done using mixtures of samples, again taken from forensically relevant sample types. Comparisons of numbers of loci and the effect of the reference populations were analyzed within this context. Specifically, the analysis was geared toward the effect of partial results on the ability to correctly analyze inclusion versus exclusion and also to assess the reference population effects on those answers. As expected, the number of loci, either as a function of sample quality or as a function of DNA concentration, had the largest effect on the ability to analyze the results while the population effects were secondary. In either case, utilization of more than approximately 50,000 SNP loci overcame the localized effects of population structure. The results were then used to determine a set of analytical parameters for the interpretation of UHD-SNP forensic DNA results.

References:

1. Homer N, Szelinger S, Redman M, Duggan D, Tembe W, *et al*. Resolving individuals contributing trace amounts of DNA to highly complex mixtures using high-density SNP genotyping microarrays. PLoS Genet 2008;4(8):e1000167. doi:10.1371/journal.pgen.1000167
2. Jacobs K, Yeager M, et.al. (2009) A new statistic and its power to infer membership in a genome-wide association study using genotype frequencies Nature Genetics doi:10.1038/ng.455

SNP, Mixture, Population Genetics

A125 Virginia TrueAllele® Validation Study: Casework Comparison

Mark W. Perlin, PhD, MD, and Kiersten Dormer, MS, Cybergenetics, 160 N Craig St, Ste 210, Pittsburgh, PA 15213; Jennifer M. Hornyak, BS, 52 Riethmiller Rd, New Wilmington, PA 16142; and Lisa C. Schiermeier-Wood, MS, and Susan Greenspoon, PhD, Dept of Forensic Science, 700 N 5th St, Richmond, VA 23219*

After attending this presentation, attendees will better understand why computer interpretation of complex DNA evidence is scientifically reliable.

This presentation will impact the forensic science community by enabling practitioners to make better use of DNA evidence already generated in their laboratory. Human review may greatly understate the evidential value of complex STR data; however, when such data are truly informative, computer reinterpretation can preserve more of the evidence, and bring that strength of match into the courtroom.

Modern criminal justice requires rapid and reliable processing of DNA evidence. Such reliability is a hallmark of admissible evidence, and encompasses sensitivity, specificity, and reproducibility. However, when confronted with complex mixtures or touch DNA, human interpretation can become a challenging task.

The result is that much evidence may be reported with an artificially low DNA match statistic, or is not reported on at all (i.e., "inconclusive"). Mathematical computing can overcome these limitations by statistically separating out contributor genotypes from the DNA mixture. These genotypes are objectively inferred by thorough consideration of the evidence data, and can be compared with reference genotypes to determine match strength.

This case study examined 111 comparisons from 92 items of evidence in 72 criminal cases involving DNA mixtures. For each item, a TrueAllele® computer provided a DNA match statistic that was reported in a supplemental case report issued by the Virginia DFS laboratory.[1] The sensitivity, specificity, and reproducibility of the computer results were examined. Comparison was made to the match information obtained by human review of the same data using Combined Probability of Inclusion (CPI) or modified CPI (mCPI). The study found TrueAllele® computer interpretation to be a reliable method for interpreting DNA mixture evidence.

Out of 111 DNA match comparisons, current mCPI human review could report on 55 of them (49.5%), finding an average match statistic of 156. TrueAllele® computer interpretation had greater sensitivity, providing 102 match statistics (91.9%) with an average value of 86.9 billion. Thus, the computer preserved more of the evidence (91.9% matches by computer, versus 49.5% manually), as well as the DNA identification information it contained (86.9 billion average match statistic, versus 156 manually).

The computer did not simply "add zeros" to the DNA match statistic. In fact, TrueAllele® statistics were lower than the corresponding human CPI values in 15 reported items. Moreover, the computer found no statistical support for a match in some cases. While, on average, TrueAllele® does find more matches and computes stronger statistics, the system objectively examines DNA evidence without any bias towards the defense or prosecution.

In addition to increased average sensitivity, TrueAllele® also maintained excellent specificity. The computer can quantify nonmatch information for exclusionary purposes through a negative log likelihood ratio (or "log(LR)"). In contrast, CPI or mCPI human interpretation methods give only positive log(LR) values, since their match statistics are never less than one. The study examined ten thousand TrueAllele® cross-case comparisons where it was expected to find no match, and found no false positives—the log(LR) was always a negative number. The average exclusionary nonmatch information in this assessment was a log(LR) of -19.693 (1 in 49.3 quintillion).

The computer objectively gave reproducible DNA match statistics. Replicate computer runs on the same evidence data showed a within-item log(LR) standard deviation of 0.305. Thus, on average, independent computer runs on the same evidence item gave statistically similar (within an order of magnitude) DNA match statistics.

DNA, whether single source or complex mixture, can provide compelling evidence that implicates criminals and exonerates the innocent. Current human review of DNA mixture data applies stochastic thresholds that can discard half the evidence and greatly understate the evidential import of what's left. As demonstrated in this casework comparison study, TrueAllele® computer interpretation more effectively preserves DNA evidence and match information. Societal safety and criminal justice may be better served by using this validated computer technology to reliably review complex DNA mixture evidence.

Reference:

1. Perlin MW, Legler MM, Spencer CE, Smith JL, Allan WP, Belrose JL, Duceman BW. Validating TrueAllele® DNA mixture interpretation. J Forensic Sci 2011;56(6):1430-47.

Computer Interpretation, DNA Mixture, Likelihood Ratio

A126 Toward Professionalism in Forensic Mathematics

Charles H. Brenner, PhD, 6801 Thornhill Dr, Oakland, CA 94611-1336*

After attending this presentation, attendees will learn basic standards for writing a methodological paper for quantitative analysis of forensic evidence.

This presentation will impact the forensic science community by increasing skepticism for forensic DNA mathematical papers, most of which fall far short of common-sense minimal standards for logical reasoned exposition.

Attendees will learn from this presentation basic standards for writing a methodological paper for quantitative analysis of forensic evidence. They will consequently have a proper scepticism for forensic DNA (in particular) mathematical papers, most of which fall far short of commonsense minimal standards for logical reasoned exposition.

DNA identification is interesting in offering, more than any other forensic area, scope for explicit mathematical treatment. There are various mathematical problems in forensic genetics beginning with straightforward linking suspect to unknown DNA profile, adding difficulties and complications such as rare haplotypes, database search or mixed sample, and identification via kinship with its many attendant intricacies. They can most clearly and usefully be dealt with through a disciplined mathematical exposition which should be precise and logical — clear statement of the problem and of assumptions, deductive progression of ideas, and justification of assumptions.

Mathematics as a tool has several potential attractions and advantages. Because mathematical writing is explicit in definitions and assumptions, it can provide clear unambiguous communication. The reader should know what is being claimed. Mathematical exposition is logical and deductive. Ideally, the reader is led irresistibly along a linear deductive path. If not irresistibly, at least the exact point of resistance is manifest. Then the reader can say "I disagree with your premise" or can argue that step D doesn't follow from step C; a productive discussion is then possible with a good chance for resolution of disagreement.

The paradigm should be: State the problem, formulate it mathematically, state premises (inevitably including a model, since this is applied mathematics), justify the premises (i.e., validate the model), derive the result. A paper which simply gives a recipe for calculation without any stated justification is professionally deficient. Yet papers meeting even these basic standards are almost non-existent.

SWGDAM instructions on rare haplotype matching don't even state a problem but instead begin by proposing how to calculate a haplotype frequency.[1] The reader who realizes that (population) frequency is not (matching) probability and that the evidential problem concerns probability, is left slack-jawed at the post with nothing with which to disagree while the paper gallops off into (irrelevant) mentions of formulas and ideas lacking not only foundation but lacking any chain of reasoning.

In the 90s, the exclusion method for mixtures was simple: If a suspect is "included" then report RMNE, calculated per-locus as the squared sum of the allele frequencies for alleles observed above 100 RFU or so. No one actually wrote down the model but the formula is simple enough that it can be reverse-engineered to deduce what the model must be: The formula assumes that all alleles of a donor will be conspicuous (e.g., >100 RFU) and "included" means all of ones alleles are conspicuous in the mixture. Obviously, this is an absurd model. That it survived and was popular and accepted for years—perhaps still—proves the importance of explicitly writing down models and explicitly deriving and justifying the consequences. *With nothing written down, nothing wrong is written down and errors are less obvious.*

The recent appearance of refining the "exclusion" method by adding a second RFU threshold suggests that RMNE enthusiasts have woken up to the folly and unfairness of the original approach.[2,3] They have not, though, woken up to the importance of models, let alone to justifying their work. Several papers give no mathematical analysis at all; only recipes which, apparently, we are supposed to trust.

The point is not that the method fails. The point is that the adherents of a method have a positive responsibility to show why it works. They have not done a respectably professional scientific job if they don't explain coherently. Otherwise the rest of us—reader, analyst in the laboratory, judge, and accused—ought to be suspicious of the validity of the method.

References:

1. SWGDAM Y-chromosome Short Tandem Repeat (Y-STR) Interpretation Guidelines. http://www.fbi.gov/.../fsc/oct2009/standards/2009_01_standards01.htm/.
2. Budowle B, *et al*. Mixture interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework, J Forensic Sci 2009;54(4):810–21.
3. SWGDAM Interpretation Guidelines for Autosomal STR Typing §3.5. Interpretation of DNA Typing Results for Mixed Samples. http://www.fbi.gov/.../codis/swgdam-interpretation-guidelines.

Standards, Forensic Mathematics, Professionalism

A127 Inter-Tissue Somatic Mosaicism in Blood, Hair, and Epithelial Cheek Cells at the ABI AmpFℓSTR® Identifiler® Loci and Its Effect on Forensic DNA Interpretation

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After attending this presentation, attendees will understand the genetic basis of somatic mosaicism, how it is distinguished from other similar genetic events, how it can be identified and confirmed in a forensic DNA profile, and its significant potential for misinterpretation when comparing DNA profiles of more than one biological source from one donor.

The presentation will impact the forensic science community by helping forensic scientists predict how often they are likely to encounter somatic mosaics in routine casework and whether their frequency is high enough to warrant changes in their procedures for comparing reference samples from suspects with crime samples and collecting samples for NDIS/CODIS database. Even if the frequency is low, several somatic mosaics are likely to be present in NDIS (>10.5 million offender profiles) or CODIS (>2 million offender profiles). The ever-growing CODIS database is currently based on inputting DNA derived from only on tissue origin. The detection of inter-tissue somatic mosaics in this study would suggest that forensic scientists should consider collecting blood, hair, and saliva samples from each offender. This becomes even more important if a partial profile from a crime scene is used to search the database. In addition, past exclusions (particularly those based on one or only a few mismatches) will need to be reassessed, possibly leading to new leads, arrests, and convictions for crimes that are currently unsolved.

Crime solving using DNA relies on the assumption that each bodily tissue of an individual exhibits the same DNA profile. Based on this prevailing assumption, positive identification of a suspect or victim can be made by matching the crime scene sample to a reference sample regardless of the origin of the bodily tissues used for the comparison. For example, a hair found on a ski mask left at a crime scene is assumed to have the same DNA profile as a blood reference sample collected from the person who left the hair. Although this assumption of inter-tissue identity is generally sound, it is not always correct because genetic mutations can cause differences in inter-tissue profiles. Somatic mosaicism is caused by mutations during embryogenesis that lead to differences in the genetic make-up of different tissues in the adult. Differences can manifest in several different ways, depending on how early the mutation occurred and whether the mutation created an allele that the individual did not already carry.

Inter-tissue somatic mosaics can be detected by collecting tissue samples from different parts of a person's body and comparing their Identifiler® profiles. The Identifiler® loci are Short Tandem Repeats (STRs), which tend to mutate more rapidly than other regions of the human genome. Therefore, it is more likely for a person to exhibit mosaicism at these loci than at most other loci in the genome. Somatic mosaicism has been well-researched in the medical community, but few studies have been completed for the benefit of forensic DNA identification.

This is the first forensic-related, multi-year research on somatic mosaicism that incorporates three distinct tissues for analysis (blood, hair, and epithelial cheek cells) from multiple donors. Currently, one out of 352 donors have been identified as a somatic mosaic. The donor's blood and saliva displayed a tri-allelic pattern (11, 12, 13) at the D8S1179 locus. The donor's hair sample also showed a small peak at the 12 allele; however, the allele was not included, because it did not cross the peak height threshold set at 20%. The results of this study indicate that somatic mosaicism can be easily detected as a tri-allelic pattern. Based on the findings, the rate of somatic mosaicism is very low (one out of 352 donors), but several somatic mosaics are likely to be present in NDIS/CODIS.

It is assumed that the DNA profile from a blood drop found at a crime scene can be successfully used to search the CODIS database, even though most of the database samples are generated from a single source (buccal cells). The detection of inter-tissue somatic mosaics in this study would suggest that forensic scientists should consider cross-tissue comparison from each offender. Further research with larger population sizes would benefit the interpretation of DNA casework.

Somatic Mosaicism, Genetic Mutation, DNA

A128 Examination of Rapidly Mutating Y-STR Loci for Increased Resolution of Common Haplotypes

Michael D. Coble, PhD, Becky Hill, MS, and John M. Butler, PhD, NIST, 100 Bureau Dr, MS 8312, Gaithersburg, MD 20899-8312*

After attending this presentation, attendees will be introduced to a set of rapidly mutating Y-STR markers. Population statistics on the specific loci examined and their utility for discriminating closely related males will be presented.

This presentation will impact the forensic science community by introducing the usefulness of a set of rapidly mutating Y-STR loci to increase discrimination among closely related males.

Y-chromosomal STR (Y-STR) testing has become an important tool for forensic investigations, especially in DNA mixture samples where low level male DNA is mixed in a high female DNA background. Presently, two commercial kits of 17 and 23 Y-STR markers are available for the forensic community and provide relatively high discrimination among unrelated individuals with a discrimination capacity greater than 97% (NIST, unpublished data).[1,2] Given the haploid nature of the Y-chromosome, a match between the evidence and the accused is evaluated in terms of how frequently the haplotype is observed in a relevant database. In addition to the limitation of Y-STR statistical results restricted to the size of the database, the current set of Y-STR markers is limited at separating related males such as fathers and sons and brothers.

Recently published rapidly mutating (RM) Y-STR loci with mutation rates from roughly 1 to 7% per meioses were evaluated at NIST using a set of unrelated population samples to determine population genetics parameters such as haplotype diversity and among a set of Father-Son samples to determine the usefulness of the markers for distinguishing related males.[3,4]

Fifteen RM Y-STR markers were organized in three multiplex reactions and amplified according to Ballantyne *et al.*[3] NIST population samples of over 600 unrelated individuals in three U.S. groups: Caucasian, African American, and Hispanics were initially tested.[5] Nearly 400 father-son samples among U.S. Caucasian, African American, Asian, and Hispanics were also tested.[6] Previously all samples have been typed for the two commercially available forensic Y-STR kits.

The study found that the RM Y-STR markers provided increased discrimination and variation among common haplotypes unresolved from the two commercially available Y-STR kits. For the father-son samples, over 21% of the samples tested exhibited at least one mutational event among the RM Y-STRs.

Conclusions: Additional Y-STR loci, especially from rapidly mutating markers, can be useful for increased discrimination among closely related males.

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Rapidly Mutating, Y-STR, Population Data

A129 Metal Ions as Forensically-Relevant Inhibitors of PCR-Based DNA Testing

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The goal of this presentation is to introduce metal ions as relevant PCR inhibitors associated with human skeletal remains to the forensic DNA community. The data presented includes sources of metal ions, their expected concentrations in forensic samples, as well as demonstrate the effects of these inhibitors on the genetic results obtained from samples amplified using PCR-based DNA amplification kits. Attendees will be given discrete examples of inhibition phenomena which can occur as a result of interactions between metal ions and DNA or PCR reagents required for testing.

This presentation will impact the forensic science community by expanding the pool of known, forensically-relevant PCR inhibitors associated with human skeletal remains to include metals commonly found in bone samples that have been exposed to the environment or to material culture. The ability to identify samples that contain inhibitors or DNA profiles, demonstrating inhibition will aid decision-making capabilities of analysts and provide a foundation for future research.

Bone samples obtained from human skeletal remains are considered one of the most challenging sample types in the laboratory. Laborious sample processing is required and it frequently yields incomplete genetic results or amplification failure. While this may be caused by and is often attributed to DNA which has been damaged, it can also occur as a result of PCR inhibition caused by the co-purification of metal ions with DNA.

Cadaver bone was obtained through the Willed Body Program at the University of North Texas Health Science Center and its metal content was determined by inductively coupled plasma-mass spectrometry (ICP-MS) by the Department of Chemistry at the University of North Texas. Elemental analysis was performed in solution mode ICP-MS to determine the concentration of analytes, including isotopes: ^{27}Al, ^{43}Ca, ^{63}Cu, ^{57}Fe, ^{60}Ni, and ^{208}Pb. ICP-MS measurements were performed on a -MS System with Autosampler. Acid digestion was used for sample preparation; approximately 100 mg of each sample was weighed out into a polypropylene test tube and dissolved in 2mL of ultra-pure concentrated HNO_3, and then diluted with 100mL of 1% HNO_3. For major and trace metal analysis, dilution factors vary between 20 and 100. All samples were measured in triplicates for statistical analysis.

In order to investigate metal ions as PCR inhibitors, a dilution series was created using certified analytical standards for aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), nickel (Ni), and lead (Pb) in solution. These standards were initially diluted to approximately 21 mM and the pH adjusted between three and five with 3 M NH_4OH and 1 M HCl; subsequent serial dilutions were prepared with DNase/RNase-free distilled water. Final sample solutions were prepared, containing 1ng of control DNA and sufficient metal to yield 7.5mM, 1.25mM, 0.2mM, 0.03 mM, 0.006 mM, and 0.001mM in the 25µL PCR reaction. Duplicate samples were amplified using the AmpF*l*STR® Identifiler® Plus (Applied Biosystems) multiplex system and a HS System. Fragment analysis was conducted via capillary electrophoresis on a genetic analyzer using

10-second injections. STR profiles, including individual allele peak heights and areas, were obtained using ID software and a 50 RFU allele detection threshold. Amplification success was determined by the average allele count, expressed as a percent of the expected number of alleles for the control DNA profile and the effect of inhibitor on the quality of the genetic results was determined by regression analysis and the calculation of Pearson's correlation coefficients.

The elemental analysis revealed isotope concentrations (µg/g) in the cadaver bone were as follows: ^{27}Al, 473-4; ^{63}Cu, 146-5; ^{57}Fe, 366-27; ^{60}Ni, 0.5-0.1; and ^{208}Pb, 165-3. The PCR inhibition studies demonstrated the effective inhibitory concentrations (mM) for amplification was: 0.001-0.006 for Al; 0.2-1.25 for Ca; 0.2-1.25 for Cu; 0.2-1.25 for Fe; 0.2-1.25 for Ni; and, Pb, 1.25-7.5. The effective inhibitory concentrations (mM) for the HS System were: 0.006-0.03 for Al; 0.2-1.25 for Ca; 1.25-7.5 for Cu; 0.2-1.25 for Fe; 0.2-1.25 for Ni; and for Pb 1.25-7.5.

Skeletal Remains, PCR Inhibition, Metal Ions

A130 Maximizing Allele Detection by Selecting Optimal Analytical Thresholds

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After attending this presentation, attendees will learn how the Analytical Threshold (AT) has a significant influence on rates of Type I (false positives) and Type II (false negatives) errors. Attendees will be introduced to Receiver Operating Characteristics (ROC) analysis as a method to compare each type of error in order to provide a method to select optimal ATs and disregard suboptimal ones.

This presentation will impact the forensic science community by providing practical guidelines on how to evaluate the signal-to-noise in order to resolve whether ATs derived from running a set of blanks can be applied during the analysis of samples amplified with low- to medium-DNA input levels and major/minor mixtures. Recommendations regarding the use of ROC will also be given.

Interpretation of DNA evidence depends upon the ability of the analyst to accurately compare the DNA profile obtained from an item of evidence and the DNA profile of a standard. This interpretation becomes progressively more difficult as the rates of Type I and II errors increase. Traditionally, ATs have been chosen to ensure the false detection of noise is minimized. However, there exists a tradeoff between the erroneous labeling of noise as alleles and the false non-detection of alleles (i.e., drop-out). In this study, the effect ATs had on both types of error was characterized. Various ATs were tested, where three relied upon the analysis of baseline signals obtained from 31 negative samples. The fourth AT was determined by utilizing the relationship between RFU signal and DNA input. The other ATs were the commonly employed 50, 150, and 200 RFU thresholds. Receiver Operating Characteristic (ROC) plots showed that although high ATs completely negated the false labeling of noise, DNA analyzed with ATs derived using analysis of the baseline signal exhibited the lowest rates of drop-out and the lowest total error rates. In another experiment, the effect small changes in ATs had on drop-out was examined. This study showed that as the AT increased from ~10-60 RFU, the number of heterozygous loci exhibiting the loss of one allele increased. Between ATs of 60 and 150 RFU, the frequency of allelic drop-out remained constant at 0.27 (+/-0.02) and began to decrease when ATs of 150 RFU or greater were utilized. In contrast, the frequency of heterozygous loci exhibiting the loss of both alleles consistently increased with AT.

Peak height ratios at each of the 7 ATs were also calculated by dividing the peak height of the smaller allele (RFU) by the peak height of the larger allele (RFU) at a locus. Those ratios were then averaged and the standard deviations from the mean were determined. The data show that as the AT decreased, the average PHR decreased, while the standard deviation remained unchanged. The average PHR at ATs of 150 RFU and an AT based on baseline analysis were 0.8 (+/-0.2) and 0.6 (+/-0.2) respectively. The drop in the mean PHR with respect to AT is suggested to be a function of the increased stochastic variation associated with the ability to detect alleles from lower quantities of DNA. This supports the proposition that forensic DNA analysis of low-template samples needs to be accompanied by guidelines appropriate for samples containing small quantities of biological material. Therefore, if a PHR threshold is used to determine the number of contributors when only two distinguishable alleles are present, this data suggests stringent PHR thresholds such as 0.7 are not optimal for samples containing <0.5ng of input DNA.

The Type I and II error rates for major:minor mixtures were also examined and showed the same general trend: lower ATs decrease the level of Type II error. However, if a significant amount of DNA from the major is present (i.e., >0.5ng), non-specific amplified product increases Type I error at low ATs which are derived from examining the baseline noise. Therefore, in order to minimize the detection error rate within the laboratory, different ATs may need to be applied to different sample types (i.e., low-template versus high-template).

Analytical Threshold, Forensic DNA, Error Rates

A131 Technical Challenges of Developing Large Multiplex STR Assays for CE Platforms

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After attending this presentation, attendees will gain a better understanding of the complexity of developing robust assays for human identification.

The presentation will impact the forensic science community by providing a better understanding of how multiplex STR assays are developed for human identification.

Multiplex STR genotyping assays using fluorescent detection and capillary electrophoresis represent the most popular method of human identification due to the highly polymorphic nature of STRs, and their small fragment size (~100-400 bp). These multiplex STR assays have a high discriminatory capacity. For example, when a complete DNA profile is obtained using the 13 CODIS loci, the probability of a chance match with a randomly chosen individual is usually less than 1 in 1 X 10^{12}. These assays have been used as supportive evidence not only to convict the guilty but also to exonerate the innocent. Given the potential consequences of STR evidence, it is important to have robust and reliable assays.

However, obtaining successful co-amplification of all STR loci in a multiplex reaction with good peak height balance between loci, specific amplification, minimal stutter, maximal non-template-dependent +A addition, and no non-specific products which might interfere with proper interpretation of a sample's DNA profile is very challenging. Furthermore, with every new primer set added to the reaction, the complexity of the PCR increases and exponentially increases the possible primer-primer and non-specific interactions. The development of an efficient multiplex PCR reaction requires careful planning and numerous tests and efforts in the area of primer design, balancing reaction components, and optimizing thermal cycling conditions. In addition, the contributions to data quality of oligonucleotide synthesis and purification, elimination of dye artifacts, and electrophoresis conditions need to be assessed.

The forensic community is concerned with many aspects of the assay such as: stutter products, non-template addition, micro variants, null alleles, accuracy, and species specificity since these can pose challenges to accurate data interpretation. They also have unique needs to handle mixtures, degraded DNA samples, PCR inhibition, and contamination since forensic casework samples do not come from a pristine, controlled environment.

Recently, the Combined DNA Index System (CODIS) Core Loci working Group in the United States published a paper stating that the number of current CODIS loci are insufficient to accommodate goals of wider data sharing internationally (greater risk of adventitious matches) and that there is a need for additional information to improve success of obtaining a profile with challenging samples, such as missing person

cases. Thus, it was recommended expanding the required minimal core loci for the database from 13 autosomal STR loci to 19, plus another sex identification marker using a Y-chromosome STR locus and three optional STR loci. This recommendation increases the potential number of markers in one multiplex assay to twenty-three.

In this presentation, an overview of the challenges typically encountered when developing highly multiplexed STR assays for human identification purposes to meet the needs of the forensic community will be presented. Examples of the problems encountered and how they were resolved will be covered. Some of the examples will cover primer design, PCR artifacts, species specificity, electrophoresis, dye artifacts, and intralocus balance.

STR, CE, PCR

A132 Development Criteria for a Next Generation Y-STR Multiplex for Forensic Applications

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After attending this presentation, attendees will understand different aspects and challenges in designing a Y-Chromosome Short Tandem Repeat (Y-STR) multiplex that can be used for multiple forensic and human identification applications.

This presentation will impact the forensic science community by demonstrating how loci selection in a multiplex might favor one application over the other. On the other hand, if enough loci are multiplexed, it might be possible to apply subsets of these loci for specific applications.

Y-STRs have been used in human identification for over a decade in paternity testing, male lineage studies, and forensic DNA analysis. Y-STRs offer certain advantages over autosomal STR analysis, such as determining the number of male contributors in a sample, multigenerational male lineage studies, and the ability to obtain a male profile in the presence of a high background of female DNA bypassing the need for performing differential extraction of sperm and epithelial cells. Y-STRs can be used for rapid exclusion of suspects. However, the discrimination power of Y-STRs is lower than autosomal STRs and non-exclusion cases may need further investigation.

In recent years, population data for several new Y-STR loci have become available in the published literature. Some of these Y-STR loci are highly discriminating and thus offer good potential for use in forensic DNA analysis. Selection of the Y-STR loci for constructing a multiplex is critical and determines its downstream applications. If the objective is to exclude close patrilineal relatives of the suspect, then markers with a high mutation rate are preferred. On the other hand, in kinship analysis, markers with high mutation rates might prove problematic. In designing a new forensic Y-STR multiplex, the inclusion of currently used Y-STRs should be considered, given that the existing Y-STR databases are already populated with profiles containing this information. New markers could be added to enhance the capabilities of already existing Y-STR multiplexes. These enhancements could combine features such as mini-STRs, the inclusion of highly discriminating markers which could allow for better differentiation of paternal lineages in populations with low Y-chromosome diversity and rapidly mutating markers.[1,2] In addition, a next generation kit should also provide improved performance for profiling of challenging samples when compared to already existing multiplexes. These enhancements include improvements to the overall amplification balance, improved resistance to inhibitors of the PCR, and shorter amplification times.

This presentation will discuss a strategy for the development of an enhanced Y-STR multiplex that combines well-known loci as well as recently characterized, highly discriminating Y-STRs into a single amplification reaction. The presentation will also address how different nomenclatures could lead to potential lack of concordance with already existing published reports in some Y-STRs. Results will show that full profiles are attainable with low levels of male DNA (below 150 pg) and that under optimized conditions no detectable cross-reactive products were obtained from human female DNA, bacteria, and commonly encountered animal species. Additionally, results demonstrate the ability to detect male-specific profiles in admixed male and female samples at ratios greater than 1:1000. The haplotype diversity and discriminatory capacity calculations for the three major U.S. population groups (Caucasians, Hispanics, and African Americans) will also be described.

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Y-STR, Forensic Casework, Paternity

A133 The Effect of pH on Electroylyte Detection of Fingermarks on Cartridge Cases and Subsequent Microscopic Examination

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After attending this presentation, the attendees will learn the basics of fingerprinting on cartridge cases and the difficulties surrounding this field. Attendees will learn about a new fingerprint enhancement technique on fired and unfired cartridge cases without any damage to the subsequent microscopic examinations.

This presentation will impact the forensic science community by providing a novel fingerprint enhancement technique that works with both fired and unfired cartridge cases without any damage to the microscopic striations.

When cartridge cases undergo the firing process, fingerprints on the cartridge can degrade. Both fired and unfired cartridge cases may be found at crime scenes, or potentially linked to crime scenes, and may contain fingerprints deposited when the firearm was loaded. The metallic, non-porous surface does not retain fingerprints well and they are further degraded by the firing process. The two most common techniques used to enhance the clarity of fingerprints on fired and unfired cartridge cases are gunblue and cyanoacrylate fuming. These methods allow for enhanced visualization on unfired cases. Forensic identification officers, using techniques that work on unfired cartridge cases often apply them to fired cartridge cases without much success. However, fired cartridge cases are still encountered and are on the rise in crime scenes, especially homicides. However, there is currently no method for the visualization of fingerprints on fired cartridge cases. A recent study performed by Jasuja and colleagues demonstrated the use of electrolyte solutions to enhance fingerprints on general metallic surfaces including zinc, aluminum, iron, brass, copper, and non-metallic surfaces such as glass and plastic.[1] This technique has not; however, been applied to cartridge cases. Thus, the purpose of this study is to test the effects of pH electrolyte solutions on the enhancement of the clarity of fingerprints on fired and unfired cartridge cases. The effect of the solution on subsequent microscopic examination of striation was also examined. Microscopic examination (MSE) can determine whether the cartridge case originated from a particular firearm; it is therefore important that fingerprint development techniques do not interfere with MSE.

The experiments involved the immersion of both fired and unfired brass cases into various pH electrolyte solutions to enhance the clarity of deposited latent fingerprints based on the study performed by Jasuja over two time periods.[1] The time of immersion was kept constant. The effect of the fingerprint enhancement technique on the microscopic features on the fired cartridge cases by the Centre of Forensic Sciences (CFS) was subsequently examined.

Fired and unfired cartridge cases were immersed in six pH solutions for 24 hours for two trials. In the first trial, the cartridge cases

were immersed into the solution three weeks after fingerprint deposition. In trial 2, the cartridge cases were immersed right after deposition. The fingerprint clarity was graded and MSE was performed. In trial 1, neutral pH was the optimal pH and trial 2, no optimal pH levels were found in fingerprint clarity enhancement on both types of cartridge cases. In terms of the first trial, it was found that neutral pH based on statistics worked the best; however, in the second trial, no optimal pH was found statistically. It was found later based on the percentage of developed fingerprints that were considered acceptable by the forensic identification officers, that the top two highest percentages were pH 1-3, 3-5. However, it was found based on these microscopic results that these two pH ranges also had the most adverse effects. The use of neutral pH level is suggested since immersion of the cartridge cases in pH 1-3, and 3-5 affects MSE. However, if MSE is not intended after fingerprint enhancements, pH 1-3, 3-5 should be used as it has the highest success rate in enhancing the fingerprints for both types of cartridge cases.

Reference:

1. Jasuja OP, Singh G, Almog J. Development of latent fingermarks by aqueous electrolytes. Forensic Sci Int 2011; 01 215-222.

Cartridge Cases, Fingerprints, Microscopic Striations

A134 The Use of mRNA Markers Allowed an Alleged Sexual Assault Casework Solution

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After attending this presentation, attendees will gain an understanding about how body fluids analysis can be useful to solve real caseworks, in particular, the use of mRNA markers as a valuable tool to identify the origin of body fluids.

This presentation will impact the forensic science community by providing results from a real casework in a relatively new field of forensic sciences with a little previous research.

Body fluid stains recovered at crime scenes are important types of evidence to forensic investigators. The first step of identifying a particular body fluid is essential since the nature of the fluid is itself very important to insure the correct handling of the sample (e.g., in mixtures from different source).

In the last years, mRNA and miRNA analysis has demonstrated to be a promising method for identification of body fluids: RNA can be isolated simultaneously with DNA, avoiding sample loss, and bypassing conventional body fluids identification methods.[1-3]

First publications showed that RNA can be isolated in suitable quality and quantity from blood, menstrual blood, saliva, and vaginal secretion.[4,5] Afterwards, a number of articles regarding the applications of RNA to further stains as seminal fluid and epithelial cells were published by several authors.

This presentation will present a case report of an attempted rape of a young woman assaulted by a friend's husband. She reported that the violence began with a "finger penetration" and then was stopped before a complete penetration was carried out. Moreover, the woman reported that during the assault, she had a fluid like discharge from her vagina (a fair amount of a reddish liquid) that stained her panties. Three days after the alleged abuse, the woman went to the emergency rescue of a local hospital for a gynecological examination, which did not detect any sign of physical violence.

The panties were collected by the prosecutor and sent to the Istitute of Legal Medicine of University of Perugia in order to evaluate the presence of biological traces attributable to the alleged assailant. Genetic analysis of the reddish liquid stain performed with the commercial DNA kit showed a mixture: the presence of Y-chromosome showed that one of the contributors was a male.

The analysis of Y-STRs performed with the DNA kit on the reddish liquid stain showed a haplotype which, compared with the suspect's haplotype, was a different one. The prosecutor questioned the woman again, she answered the questions, underlining that she did not have any sexual intercourse with any other person the day of the alleged violence. She also reported that five days before the alleged violence she had unprotected sexual intercourse with her partner and had taken a progestin pill.

Thereafter, an analysis of mRNA was performed on the reddish liquid stain, using two different markers: MMP11 (present in the endometrial cells and commonly used for the diagnosis of menstrual blood) and PRM2 (specific for sperm cells).

The analysis of the mRNA was positive for PRM2 and negative for MMP11. The presence of sperm was suggested. Therefore, an analysis of Y-STRs on the victim's boyfriend was performed. The obtained Y-haplotype matched with the Y-haplotype achieved from reddish liquid stain. Probably the reddish fluid discharge from the vagina was the consequence of progestin pill consumption.

References:

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5. Bauer M, Patzelt D. Identification of menstrual blood by real time RT-PCR: Technical improvements and the pratical value of negative test results. Forensic Sci Int 2008;174:54-58.

Body Fluids, mRNA Analysis, Sexual Assault

A135 Analysis of Fluoro-Phenethylamine Regioisomers Using Electrospray Ionization-Mass Spectrometry and Capillary Electrophoresis

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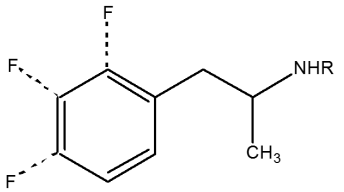
After attending this presentation, attendees will become aware of how ESI-MS and CE techniques can be used to distinguish between 2-, 3-, and 4-fluoroamphetamine and 2-, 3-, and 4-fluoromethamphetamine.

This presentation will impact the forensic science community by providing awareness of alternative methods for analyzing ring regioisomers of controlled substances.

During the past couple of years, local, state, and federal forensic chemistry laboratories have encountered a great array of new designer chemicals. The new compounds are available through the clandestine drug market and have been designed with the intention of circumventing legal regulations. Laboratory submissions containing these new compounds present significant challenges to analysts and laboratory managers due to both the lack of reliable sources of reference materials and the inability to use routine laboratory techniques like gas chromatography-mass spectrometry (GC/MS) to unambiguously distinguish potential chemical variations in the structure of the compounds.

Two types of new designer compounds recently encountered are fluoroamphetamine (FA) and fluoromethamphetamine (FMA), which may be considered structural analogues of controlled phenethylamines. As observed in the structures below, there are three possible phenyl ring regioisomers for each one of these compounds, depending on the location of the fluorine atom. When the fluorine atom is located in position two, three, or four, the resulting compounds are the *ortho*, *meta*, or *para*-fluoro-phenethylamines, respectively. It is believed that

replacement of the hydrogen atom by fluorine facilitates passage through the blood-brain barrier, as the substitute atom increases the lipophilicity of the compound.[1]



R = H (amphetamine) R = CH_3 (methamphetamine)

Analysis of FA and FMA using routine GC/MS conditions results in similar retention times and electron ionization (EI) fragmentation patterns for all three positional isomers of each compound. That is, the unequivocal distinction between 2-FA, 3-FA, and 4-FA, and between 2-FMA, 3-FMA, and 4-FMA cannot be accomplished solely by GC/MS analysis. This presentation will describe application of the techniques of electrospray ionization mass spectrometry (ESI-MS) and capillary electrophoresis (CE) to the analysis of FA and FMA regioisomers.

Analysis of 2-FA, 3-FA, and 4-FA using ESI-MS results in protonated pseudomolecular ions at m/z 154, consistent with the molecular weight of 153 Da. The analogous ions at m/z 168 were observed during analysis of the FMA regioisomers. Collision-induced dissociation (CID) experiments were designed in order to investigate the possibility for distinguishing the regioisomers from each other. To achieve this, the CID conditions were methodically varied between 20% and 30% collision energy, and MS^2 and MS^3 fragmentation data were collected for each of the six regioisomers. Based on the fragmentation patterns observed, it can be concluded that 2-FA and 3-FA cannot be distinguished from each other. However, the fragmentation pattern observed for these two regioisomers can be clearly differentiated from that obtained for 4-FA. This pattern of distinction is statistically reproducible and also repeated within the FMA regioisomer series. That is, 2-FMA and 3-FMA result in similar MS^n fragmentation spectra, which then can be distinguished from that of 4-FMA.

Analysis of FA and FMA regioisomers using routine non-chiral CE experimental conditions resulted in similar migration times for all six regioisomers. Under chiral analysis conditions, the migration times observed changed, but are only slightly distinguishable. However, the use of 2-OH-β-cyclodextrin as a buffer additive did result in noticeable differences between the enantiomeric pairs of each compound. A reproducible pattern of separation is observed which can be used to distinguish between the *ortho*-, *meta*-, and *para*-phenethylamines. The resolution between the *d*- and *l*- enantiomer peaks is observed to increase as the position of the fluorine is changed from four to three to two; that is, as the fluorine atom moves closer to the alkyl chain. For 4-FA and 4-FMA, the enantiomers are practically co-migrating; for 3-FA and 3-FMA the peak separation increases and the presence of 2 enantiomers is evident, although baseline separation is not complete. For 4-FA and 4-FMA, the resolution values measured are twice those observed for 3-FA and 3-FMA, respectively.

This presentation will also include application of the above described ESI-MS and CE techniques during the analysis of multiple unknown case samples. This material included in this presentation is expected to be of interest to other forensic chemistry analysts and laboratory personnel involved in the analysis and evaluation of controlled substance analogues.

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Regioisomer, Analogues, Controlled Substance

A136 Micro-Raman Mapping of Dissimilar Inks on Paper

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After attending this presentation, attendees will be presented with an example of identifying the order of ink deposition where lines cross by micro-Raman mapping using two types of maps, surface, and depth slice, providing a visual interpretation of the analytical data that is easy for juries to understand.

This presentation will impact the forensic science community by demonstrating the use of micro-Raman mapping as a visual technique well suited for courtroom presentations.

When documents are altered, the order of ink layer deposition where lines cross is an important determination confronting questioned document examiners. Knowledge of the top layer suggests the intent of the alternation and knowledge of the lower layer provides information about the document's original content. Together, this information helps establish motive, may suggest a suspect, and establishes a timeline of the crime.

Conventional ballpoint pens were tested by making a figure of a cross with single lines of different inks. Reference spectra were acquired at various locations on the inked legs of the cross where lines of individual inks existed, and for paper at adjacent locations near the inked lines. At the microscopic level, paper composition is inconsistent and ink coverage is inconsistent and incomplete. These difficulties were overcome by averaging several repeated scans at every test location to assure instrument accuracy, followed by averaging all scans of each individual ink and paper to compile representative standard spectra of each component. The same sampling scheme was used in the area where lines crossed.

Micro-Raman analysis does not destroy the sample. Confocal optical design limits the depth of focus to very thin layers, allowing small volumes to be analyzed without interference from the surroundings. Raman excitation was 785nm and spectral response was measured from 850cm^{-1} to 3200cm^{-1} or from 1250cm^{-1} to 1750cm^{-1}. Other instrument settings were 30 second exposure time, 10% laser power, and two accumulations at either 20x or 50x magnification at high confocality.

Maps are like digital photographic images composed of cells (pixels) that hold both the Raman spectrum and photographic information at that location. Steps between cells can progress at 1.0 micrometer increments in either the x or y horizontal directions to produce two-dimensional (2D or x,y) surface maps; or vertically (y direction), to produce depth slice maps that appear as vertical cross sections above and/or below the paper surface.

To show ink locations on the mapped surface, standard reference spectra of paper and each ink were assigned a false color and matched to the spectra in each mapped cell. The location of each component was visualized by redrawing the map with the assigned false colors.

False colored maps require little technical explanation and are easily understood, allowing juries to draw their own conclusions. This frees the expert of the need to express his own conclusion that judges sometimes disallow because it is held to be "subjective."

Micro-Raman Mapping, Ink Analysis, Document Alternation

A137 Raman Spectroscopy as a Non-Destructive Technique to Differentiate Circulatory and Menstrual Blood

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After attending this presentation, attendees will have learned about the significance of Raman spectroscopy as a non-destructive technique in the differentiation of body fluids at a crime scene.

This presentation will impact the forensic science community by learning a more effective and rapid technique for body fluid identification at a crime scene. The development of portable handheld Raman instruments further allows the investigator to analyze samples in the field to obtain rapid results prior to collection of evidence.

The versatility and non-destructive nature of Raman spectroscopy has lead to its widespread use for rapid analyses in forensic science. The development of portable handheld Raman instruments further allows the investigator to analyze samples in the field to obtain rapid results prior to collection of evidence. Raman is already used to identify fibers, drugs, explosives, lipsticks, ink, paint, bones, fingerprints, and condom lubricants. The benefits of Raman spectroscopy, besides its main characteristic of being non-destructive, include reagent-free and minimal sample preparation, little interference from water, and a sample size as small as several picrograms. In addition, results may be obtained through transparent packaging, thus allowing containment of potentially hazardous or biohazardous materials. Recent research has shown the potential for Raman spectroscopy to definitively and non-destructively identify and differentiate common body fluids including semen, saliva, vaginal fluid, sweat, and blood. Raman spectroscopy also has the potential to distinguish, non-destructively, human, canine, and feline species by their blood spectra using statistical analysis. Blood is the most common body fluid found at a crime scene and, in certain cases, the ability to distinguish menstrual blood from circulatory blood is desirable and may be critical. Current methods for identification of menstrual blood include the use of microscopy, lactate dehydrogenase isozyme identification, Messenger Ribonucleic Acid (mRNA) and Microribonucleic Acid (miRNA) profiling, real time-PCR, and identification of the products of fibrinolysis. These methods are complex, destructive, expensive, and have the potential for false negative results. Current methods for identification of circulatory blood are heme-catalyzed screening tests, which often involve a color change. These tests can be performed at the crime scene, but can have false positives and consume the sample. This research investigates the use of Raman spectroscopy as a tool to rapidly differentiate between circulatory and menstrual blood. Preliminary results have identified the main components of liquid and dried blood as well as investigated the spectra of blood on various substrates. Results show no difference in the spectra across genders and a time study of blood showed an increase in peak intensity of the component fibrin with time. The main components of liquid blood were hemoglobin, fibrin, glucose/L-tryptophan, and L-tryptophan/L-phenylalanine. The same main components were observed in dried blood. However, significant differences in peak patterns were present in liquid and dried blood due to coagulation, which included changes in peak morphology and an increase in peak intensity. The major physiological differences between menstrual blood are related to the ability of blood to clot and the presence of clotting agents, such as fibrin, in the blood. The ready detection of these agents in circulatory blood using Raman spectroscopy shows promise for its further use to identify menstrual blood. Menstrual blood has a non-clotting agent that breaks up clots and therefore affects the fibrin level present. In this study, menstrual blood was collected on gauze pads from female volunteers during two different menstrual periods. The same volunteers provided circulatory blood via a conventional blood draw. Differences in fibrin levels shown by Raman spectroscopy may be linked to the origin of the blood. Subtle differences in spectra were resolved using Chemometrics, specifically R, to show clear differentiation between menstrual and circulatory blood.

Raman, Blood, Fibrin

A138 Separation and Identification of Synthetic Cathinones Using GC/MS, GC-QQQ/MS, and ESI-IMS/MS

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After attending this presentation, attendees will learn the use of ion mobility as a separation technique coupled to a mass spectrometer for the analysis of synthetic cathinone and the differences in ionization (ESI vs CI vs EI) in fragmentation and identification of these analytes of interest.

This presentation will impact the forensic science community by making it possible to unambiguously separate the cathinones with IMS faster than with GC/MS, while also preserving the molecular ion due to the soft ionization using ESI.

Cathinone is the main component of the *khat* plant which produces a stimulating effect similar to amphetamines.[1] The ease of online availability of a number of the *synthetic cathinones*, also known as legal highs or bath salts, have led to increased use and abuse over the past few years.[2] In this study, six synthetic cathinones, recently scheduled as Schedule I controlled substances in Florida as of July 2012, 4-methylmethcathinone (mephedrone or 4MMC), 3-fluoromethcathinone (flephedrone or 3FMC), 4-methylethcathinone (4MEC), 4-methoxymethcathinone (methedrone or bkPMMA), 3,4-methylenedioxymethcathinon (methylone or bkMDMA), and 3,4-methylenedioxypyrovalerone (MDPV), were analyzed using a commercially available electrospray ionization-ion mobility spectrometer-mass spectrometer (ESI-IMS-MS), a gas chromatograph-mass spectrometer (GC/MS) using electron ionization (EI), and a GC/MS Triple Quadrupole (QQQ) with both EI and chemical ionization (CI) modes. One of the advantages of using the softer CI and ESI ionization sources is the creation of molecular ions of the easily fragmented compounds and, in the case of ESI, the ability to analyze nonvolatile compounds and ionize compounds in the liquid phase.[3] A fast and selective method is reported that can be used to unambiguously identify this increasingly important class of drugs. A high-resolution IMS was used to separate mixtures of these synthetic cathinones within less than 20ms. The limits of detection were determined and good separations from two-compound mixtures were achieved with typical concentrations of MDPV and other compounds (20:100ppm), and methylone and other compounds (20:100ppm) using ESI-IMS-MS. The analysis of these cathinones was also carried out using GC/MS, which is currently the standard technique used in forensic laboratories, to compare with ESI-IMS-MS. In addition, GC-QQQ-MS with EI and CI modes was used to determine the identification of fragmented ions from these cathinones. The analysis of several actual seized police case samples was also performed using the same instruments to illustrate the utility of the developed IMS-MS and GC-QQQ-MS methods.

These methods, reported for the first time for the drugs listed, make it possible to unambiguously separate the cathinones with IMS faster than with GC/MS while also preserving the molecular ion due to the soft ionization using ESI.

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Synthetic Cathinones, ESI-IMS-MS, GC-QQQ-MS

A139 Determination of Organic Gunshot Residue With Solid Phase Microextraction by GC/MS

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After attending this presentation, attendees will become familiar with how Solid Phase Microextraction (SPME), coupled with Gas Chromatography-Mass Spectrometry (GC/MS), can be used to effectively detect organic gunshot residue (OGSR) from articles of clothing.

This presentation will impact the forensic science community by providing a quick and efficient method to detect OGSR on a suspect's clothing. Additionally, while utilizing equipment currently present in most labs, forensic labs will be presented with an alternative method to determine if an individual has fired a gun.

Traditionally, GSR has been evaluated by elemental analysis of inorganic particles such as lead, barium, and antimony by the technique of Scanning Electron Microscopy with Energy Dispersive X-ray analysis (SEM-EDX). This method has provided the benchmark for GSR analysis within the legal system for decades. Although the technique of SEM-EDX is very effective, it also has the disadvantage of being time consuming and labor intensive.[1]

As times change, the compositions of ammunitions are also evolving. More environmentally friendly formulas remove lead, and replace it with other less toxic elements such as aluminum.[2] The traditional method of analyzing GSR by SEM-EDX, which relies on the presence of these inorganic elements, is becoming obsolete as manufactures change the composition of their ammunitions to more environmentally sustainable formulas. This has placed the forensic community at a disadvantage when it comes to GSR analysis, but at the same time also gives new opportunities to create alternative methods to solve such problems.

The goal of this study is to determine if OGSR can be captured by SPME and analyzed by GC/MS to establish if an individual fired a gun. OGSR can originate from many components of ammunitions including primers, propellants, and stabilizers—all of which contain different compounds classified as OGSR. Some of these compounds include diphenylamine (DPA), N-nitrosodiphenylamine, ethyl centralite (EC), and methyl centralite (MC). The compounds ethyl and methyl centralite are both unique compounds to OGSR, and can be used as an identifier compound to place a suspect at the scene of a shooting.[3]

For this research, articles of clothing were collected in sealed cans for transportation and analysis. The OGSR was moved from the clothing into the headspace of the can by applying heat in an oven. The SPME fiber was then exposed to the headspace to extract the OGSR. After the headspace extraction, the OGSR was desorbed from the SPME fiber into the heated injection port of the GC/MS for analysis. A 75µm Carboxen-PDMS (Supelco Bellefonte, PA) SPME fiber was chosen to perform the extraction with a Shimadzu GC/MS-QP5000 (Kyoto, Japan) for the analysis. To make the analysis more selective, a selected ion monitoring (SIM) method of known OGSR compounds was used to perform the analysis. Test firings/samples were conducted at a local shooting range.

Preliminary data has demonstrated that a cloth doped with OGSR compounds DPA, EC, and 2,4-dinitrotoluene can be extracted and analyzed with SPME-GC/MS. Initial findings have also shown that when a gun is discharged, a cloud of OGSR does adhere to cloths placed around the shooter, with some of these compounds being extracted when analyzed. Additional experiments are being conducted to determine if a correlation is present between certain compounds and individual brands of ammunitions. The preliminary data has shown that using SPME-GC/MS as the means of extraction and detection will provide a fast and accurate method for the analysis of OGSR evidence.

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GC/MS, Organic GSR, SPME

A140 Determination of Useful Yields of Forensically Relevant Compounds by Direct Analysis in Real Time Mass Spectrometry (DART/MS)

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After attending this presentation, attendees will be familiar with the concept of useful yields and their relevance to the forensic community, while also discussing the topics of useful yields for a number of forensically related compounds, including explosives, narcotics, bank dye, and lubricants.

This presentation will impact the forensic science community by giving an in-depth understanding of useful yields and how they can be improved to better understand the limit of detection and limit of quantification of mass spectrometry analysis. Furthermore, the effects which instrument parameters have on the useful yields of the compounds will be explored. Comparison of the useful yields of the DART®-MS technique to other mass spectrometry techniques will also be discussed.

Useful yield is a term which is not widely used in the Ambient Pressure Ionization Mass Spectrometry (API-MS) realm. It has; however, been utilized in the Secondary Ionization Mass Spectrometry (SIMS) area of study. The definition of useful yields is the ratio of the ionized molecules to the amount of molecules originally present in the sample. This measurement allows for a way to measure the efficiency and practical yields of a given substance through analysis on a specific technique with specific settings. Useful yields can also be used to compare two techniques which are difficult to compare otherwise. By understanding the useful yields it may be possible to determine which technique would provide the best response for a given analyte or class of analytes.

To obtain a useful yield, it is crucial to have a precise understanding of the number of analyte molecules present in the sample. In order to obtain an accurate number, two methods of sample deposition can be used. The first method is a microsyringe, which will deposit a relatively accurate amount of analyte to a substrate. The second and more precise technique is piezoelectric inkjet printing, which allows for the printing of microdroplets of solution onto a surface. Using this technique, it is possible to obtain 1% repeatability and ±10% accuracy in sample deposition.

Useful yields were determined for a number of compounds on an AccuTOF™ DART® mass spectrometer. The DART® source is a commercial-available API-MS technique which is gaining momentum for routine use in casework, and, therefore, is a prime technique for this study. Since there are several different parameters which affect the signal, the useful yields under a number of different parameters were studied. These parameters include gas temperature, orifice voltage, and the addition of a dopant. How much of these factors influence the useful yield is discussed. The analytes which are examined include explosives, narcotics, bank dye, and lubricants. Initial results on the useful yields of explosives show that about one in every 10^8 – one in every 10^9 molecules are ionized and detected. This compares closely to results for other API-MS techniques, including Desorbtive Electrospray Ionization (DESI) and Low Temperature Plasma (LTP). Useful yields from DART®-MS are also compared to both vacuum-based techniques and chromatographic techniques such as SIMS and GC/MS.

DART®-MS, Useful Yields, Explosives

A141 Analyzing Lateral Diffusion of Fingerprint Constituents as a Function of Time Since Deposition

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After attending this presentation, attendees will be aware of how imaging mass spectrometry could be used as a technique to monitor the diffusion of fingerprint constituents as a function of time since deposition. Through the ability to image several different components, the potential for this technique to be able to determine an approximate age of a fingerprint will also be presented.

This presentation will impact the forensic science community by introducing a unique and innovative application of imaging mass spectrometry, more specifically, how imaging mass spectrometry can be used to better understand fingerprints, concentrating on chemical changes in a fingerprint.

Though the imaging of fingerprints has been reported previously using mass spectrometry techniques, the use of the technique as a potential way to monitor the age of fingerprints has not been discussed. Through initial studies, it has been shown that the relative location of certain components in a fingerprint change as the fingerprints age. The general trend appears to be that a number of constituents appear to diffuse out of the ridges of the fingerprints and into the valleys. Furthermore, there appears to be a threshold time after which these compounds have completely diffused and the ridges and valleys of the fingerprint cannot be differentiated using chemical imaging. However, even though the chemical image is indistinguishable, ridges are still optically visible.

The technique which is used to obtain the images is a Secondary Ion Mass Spectrometer (SIMS). The SIMS technique is a high vacuum technique in which a primary ion species—in this case either bismuth or fullerene ions—is accelerated toward a sample and, upon interaction, causes the ejection of secondary ions, which are characteristic of the sample. SIMS is a soft ionization technique, and readily produces the molecular ion of nearly all chemical species. Furthermore, this technique has already been shown to readily detect a number of different sebaceous and eccrine components of a fingerprint. SIMS also provides several benefits over other types of imaging mass spectrometers, such as ambient pressure ionization mass spectrometry, the most important of which is the ability to provide chemical images with spatial resolution of 1µm or better. This is a level of spatial resolution which is difficult to obtain using techniques such as DESI-MS. This high-resolution technique also allows for the simultaneous imaging of all chemical signals present in a fingerprint, which enables monitoring and comparison of a number of different fingerprint components simultaneously.

Initial studies have shown a number of fingerprint constituents diffuse from fingerprint ridges over varying time scales. These changes have been noted to occur over several months, which may provide a way to tell differences in fingerprints over longer times cales than just days to weeks. While changes in the long time scale have been identified, experiments are also being completed to discover changes which provide a way to date on a shorter time scale. Finally, the effects of sample substrate and environmental exposure conditions will be analyzed. An evaluation on the practicality of the technique will also be presented.

Fingerprints, SIMS, Chemical Imaging

A142 Revisiting Glass Fracture Examination and Interpretation in Forensic Casework

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After attending this presentation, attendees will know about the scientific terminology used in glass fracture analysis, how to avoid common pitfalls when determining the direction of force, and how to distinguish between various types of glass fractures.

This presentation will impact the forensic science community by introducing the proper scientific terminology for describer fractures in glass.

During crime scene investigations and reconstructions involving broken or fractured panes of glass, it is often of vital importance whether or not a location or vehicle was entered from the inside or outside. In burglary investigation, whether a pane of glass was shattered with a tool, struck with a baseball bat, or cut with a glass cutter is crucial information for the investigating detective to know. Whether the glass window was broken or cut from inside the premises or outside the premises is crucial to the burglary investigation. Investigations involving arson can often be advanced by knowing if the glass windows were broken by thermal radiation, a projectile, or some other type of physical force. Events involving firearms, bullet holes, and bullet trajectory can often benefit greatly from a thorough and complete scientific evaluation of the glass fractures. The sequencing of bullet holes is often very important to reconstructing a crime scene or event. Determining which bullet hole was made first, or whether a bullet hole was made from outside-in or inside-out of the premises is often crucial to an investigation. In leaving the scene of accident investigations involving fractured windshields, studying the scattered windshield can often help determine who was driving the vehicle and who was a passenger, where the occupants were siting, how fast the vehicle was going, and much more. Such issues have been studied and discussed by forensic scientists for nearly a century, and many have published their work in the forensic literature.[1-12] Unfortunately, although much of the published information is useful and scientifically accurate, the nomenclature used for fracture marks is often confusing, and has been frequently misused in the scientific literature.[13] In the mid 1980s, two forensic scientists addressed some of these issues; however, more work stills needs to be carried out.[14]

This study delves into the scientific methods and terminology used by those material scientists that study the fractography of brittle materials such as glasses and ceramics. The basic definitions of natural and synthetic glasses, as well as the various types of commercial glass, are presented and discussed. Next, their physical, optical, and chemical properties are reviewed in relationship to how and why these brittle materials fracture. The brittle nature of glass, its elasticity, tensile strength, and the scientific laws and phenomena explaining its behavior are examined and discussed in detail.[15-20]

The proper scientific terminology for commonly used terms such as craters, splintering, rib marks, hackle marks, radial lines, and concentric lines are offered, depicted, and covered in detail. Some common pitfalls in applying the right, rear, radial rule for determining the direction of forces are also advanced. Several case studies are given which illustrate and discuss the scientific laws, and terminology used by the fractography community for describing glass fracture phenomenon and showing how these laws and terms can be applied in forensic casework.

Finally, the goals of this research are to help right these issues in at least the forensic community and to further the use of glass fracture analysis in forensic casework meeting the challenges of the NAS Report on Forensic Sciences.

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Fractography, Hertzian Conoid, Wallner Lines

A143 A Survey of Forensic and Law Enforcement Professionals on Synthetic Drug Trends: A One Year Follow-Up

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After attending this presentation, attendees will gain an understanding of the changes in trends of synthetic drugs throughout the United States that occurred over the past year.

This presentation will impact the forensic science community by demonstrating the change in trends for synthetic drug samples in a one-year period. Synthetic drug compounds are continuously being introduced into the United States; therefore, staying current on changing trends is important for police officers and forensic scientists.

A survey was given to forensic science and law enforcement professionals participating in online continuing education courses during 2011. Due to the rapid changes in synthetic drug compounds and related legislation, a follow-up survey was performed one year later to observe changes in trends in that period. The original survey, as well as the follow-up survey, consisted of the questions listed:

1. In what state do you work?
2. Do you work for a local or state agency?
3. What is your job title?
4. Have you encountered synthetic drugs samples in your position?
5. What synthetic drug compounds are you specifically seeing?
6. How often are you working with synthetic drug samples?
7. Do you perform testing in-house for synthetic drugs?
8. If yes, can you elaborate on your testing protocol?
9. If no, what is the protocol for synthetic samples in your agency?
10. What further information can you provide regarding synthetic drugs?

The original survey showed that 79% of those surveyed had encountered synthetic drug samples in their job position. Of those surveyed, the majority were testing synthetic drug samples monthly, although some were testing samples daily.

The follow-up survey had one additional question added in order to record the number of professionals who participated in related surveys, courses, and articles in order to determine how much information and/or questions they have received in the last year. The purpose of the latest survey is to gather the most up-to-date data possible and present this information to the forensic community. The current survey data will be compiled through January 31, 2013, allowing for the most current data to be presented. Comparing the original survey and the current survey will aid in demonstrating the rapid pace at which these types of compounds develop.

The original survey was given in order to observe overall trends in synthetic drugs from the forensic professional's view, while the follow-up survey will provide the forensic community with a more detailed explanation of what has occurred over the last year in the synthetic drug market. More individual cities and states are enforcing their own bands on specific compounds and groups of compounds; therefore, observing recent trends is important. Synthetic drugs are unable to be classified as easily as other illicit drugs due to the vast number of compounds; therefore, identifying trends may aid in the process of uniformly banning synthetic drugs across the country. Not only will the results demonstrate what is currently being observed throughout the country, but what may be seen in the future. Unlike traditional illicit drugs, synthetic drugs are continually being developed, which causes problems for forensic laboratories due to the lack of identification testing methods. With the rapid development of this group of drugs, published data may quickly go out of date, which is why an up-to-date, one-year comparison survey can benefit the forensic community. Traditionally, a one-year follow-up survey may not present enough data to show change, yet with a market such as synthetic drugs, a year is a large enough time frame for significant changes to be observed.

Synthetic, Drugs, Trends

A144 The Evaluation of Chemical Interferences in Forensic Tape Analysis From FreeZ-It®, Un-Du®, and Super Glue® Fuming

Katie Villarreal, MSFS, 4930 N 31st St, Forest Park, GA 30297*

After attending this presentation, attendees will have a better understanding of the possible chemical interferences contributed from three techniques commonly used by latent print examiners in the beginning stages of latent print examinations on pressure-sensitive tapes.

This presentation will impact the forensic science community by clarifying potential chemical interferences that latent print processing techniques can have on the analysis of pressure-sensitive tapes. The collaboration between trace evidence examiners and latent print examiners in regard to handling the forensic examinations of these tapes is critical in promoting the development of probative information.

Pressure-sensitive tapes are commonly encountered in forensic science casework in the form of restraints, ligatures, blindfolds, mail fraud, and in improvised explosive devices. The potential exists, in cases involving pressure-sensitive tapes warranting both trace evidence examinations and latent print examinations, for one's examination to hinder or impede the other's examination(s). This potential "trade-off" is case specific and must result in close collaboration between examiners from each discipline and investigators and/or litigators. Is there a workflow that would allow for both disciplines to minimize the potential effects that each examination can have on the other? Does the processing conducted by the United States Army Criminal Investigation

Laboratory's (USACIL) Latent Print Branch to protect any potential latent print(s) and DNA evidence hinder trace evidence chemical comparison examinations? Is it pertinent that trace evidence sample prior to any latent print processing or can latent prints take steps to preserve any potential latent prints (and DNA evidence associated with latent prints) up to the step of Super Glue fuming?

This research examined the possibility of minimizing the "trade-offs" that exist between trace evidence and latent prints in regard to the examination of pressure-sensitive tapes by determining if there are any chemical interferences observed in tape processed with FreeZ-It®, Un-Du®, and Super Glue fuming (cyanoacrylate) for three types of tape commonly encountered in casework (duct tape, vinyl/electrical tape, and clear packing tape). The processed tapes and neat samples were analyzed by Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Fluorescence (XRF). All treated and neat samples were analyzed by Attenuated Total Reflectance (ATR). In addition to ATR, the duct tape samples were also evaluated by transmission FTIR (microscope) allowing for evaluation between the techniques. All treated and neat samples for the three different types of tapes were analyzed by XRF in triplicate. Statistics were applied to the XRF data to assist in determining if any significant differences occur in the spectral data.

There is evidence that suggests chemical interferences are present in tapes processed with super glue and analyzed by ATR. Also, there is evidence that supports significant differences in elemental data in duct tape adhesive and duct tape backing treated with FreeZ-It®, Un-Du®, and Super Glue when compared to the neat samples.

Trace Evidence, Latent Prints, Tape

A145 Ball Footprint Classification System

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As a result of noticing a low success rate for identifying school children from their infant footprints, the goal of this presentation is to help attendees understand the importance of footprinting newborns correctly in order to obtain a positive identification.

This presentation will impact the forensic science community by assisting officials in identifying children in various cases. By implementing a more thorough methodology in footprinting newborns, the amount of misidentifications can be decreased.

There has been a low success rate for identifying school children from their infant baby prints. Baby prints have historically been used by hospitals as souvenirs for parents. These prints, while valuable in the wealth of information that can be obtained from them, are often taken incorrectly; many prints are obtained from those who have little training in fingerprinting an individual. The art of fingerprinting an individual is key when it comes to footprinting newborns because of the similar technique needed to successfully obtain a suitable print. Even the footprints obtained from twenty newborns at five different nurseries that are known to provide maximum detail only had 11% of prints that were deemed adequate enough for identification by an expert analyst. Research has shown that the development of the arch of the foot around the ages of four and five, results in an unsuccessful identification in children.[1] A study conducted by the California Department of Justice saw that out of 50 subjects, they could only identify a handful.[2] Low success rates have also been found when attempting to identify children using a co-classification system where a researcher utilized two methodologies in classifying footprints.[3] Research conducted from this study has indicated that utilizing a two-dimensional method that focuses solely on the ball of the foot would result in a higher match rate. Similar to the principle of fingerprinting, instead of using the whole hand to classify an individual, the study will show that the imprint of just the balls of the feet will provide a suitable and accurate way for classifying an individual from infancy to adulthood. Creating a classification system solely using the ball of the foot will yield a higher match rate than examining the entire foot. Volunteers have provided their baby footprints for analysis in this project. Additionally, current prints were obtained from volunteers through the ink rolling method for comparison and for the creation of a database. Through comparison of both baby footprints and their adult counterparts, unique characteristics for each individual were dominantly seen in the area of the balls of their feet, but as we moved to the sole and the heel of the foot, unique characteristics were not as prominent. This proves promising in the development of a classification system that would focus mainly on the ball of the foot. Data gathered from this research could be the foundation for a national database in order to help identify children when other methods of identification are unavailable due to cost, lack of DNA exemplars, or time constraints. Implementation of this proposed system would rely heavily on educating numerous hospitals in order to obtain suitable prints for future use in the identification of children as they age.

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Footprint, Identification, Classification

A146 Forensic Mitochondrial DNA Analysis of Human Hairs After Exposure to Radiation

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The goal of this presentation is to inform of the effects that radiation type and dosage have on Mitochondrial DNA (mtDNA) analysis of human hairs, allowing one to judge in which situations hair evidence is likely to remain useful for mtDNA analysis, and, therefore, should be collected.

This presentation will impact the forensic science community by understanding how the effects of radiation on the mtDNA analysis of hairs will prevent unnecessary radiation exposure to the individuals that would collect the hair evidence. This presentation will give decision makers more data to help determine whether or not the exposure of evidence to certain levels and types of radiation justifies the potential health risk for the evidence to be collected.

The objective of this presentation is to inform the forensic community of the effects that radiation type and dosage have on Mitochondrial DNA (mtDNA) analysis of human hairs. This will allow one to evaluate in which situations hair evidence is likely to remain useful for mtDNA analysis and; therefore, should be collected. In this way, knowing the effects of radiation on the mtDNA analysis of hairs will prevent unnecessary radiation exposure to the individuals that would collect the hair evidence.

Studies have shown the effects of DNA recovery from gamma irradiated human blood and paper[1] and the effects on nuclear DNA profile analysis from gamma and alpha irradiation of human blood, bone, saliva, and a genomic standard.[2] Here human hairs were irradiated with varying levels of gamma, neutron, beta, and alpha radiation in an attempt to determine the effect of these exposures on mtDNA analysis.

The hair samples were exposed to various levels of the four different types of radiation at the Savannah River National Laboratory, South Carolina, in replicates of five hairs per treatment for each of three individuals. Radiation doses included gamma ($5x10^4$ – $9x10^8$ rad), beta ($5x10^1$ – $1x10^3$ rad), alpha ($5x10^7$ - $5x10^{11}$ MeV), and neutron ($1x10^{10}$ – $1x10^{13}$/cm^2). Hair samples exposed to the highest radiation doses within each radiation treatment were initially extracted, mtDNA hypervariable

regions I and II were amplified, the regions cycle-sequenced and sequenced, and finally the data were analyzed. These procedures followed the FBI Laboratory Mitochondrial DNA Unit Mitochondrial DNA Analysis Protocol (revision 5), with one exception: only one HL60 positive control sample and one negative control sample were amplified and analyzed per mtDNA region, per radiation treatment. When extraction, amplification, or sequencing of mtDNA was not successful within an irradiated sample dose, analysis of additional hairs was attempted. If mtDNA from the additional hairs was not successfully extracted, amplified, and sequenced, then hairs exposed to the next lower dose of that radiation type were analyzed. DNA extraction was successfully attempted on all hairs exposed to the highest dose of each radiation type, with the exception of the gamma $9x10^8$ rad treatment. Hairs exposed to that level of gamma radiation were physically degraded and not suitable for extraction.

Once mtDNA sequences were obtained from irradiated hairs, the mtDNA sequences were compared to mtDNA sequences from nonirradiated hairs from the same individual. These comparison hairs were subject to the same conditions as the irradiated hairs minus the radiation exposure (brought from the FBI laboratory to the Savannah River National Laboratory and back at the same time and stored in the same conditions).

DNA amplification was successful for all hairs exposed to each radiation treatment that were suitable for extraction, with the exception of the $1x10^6$ rad gamma radiation treatment. Only one of five hairs exposed to the $1x10^6$ rad level of gamma radiation produced extracted DNA with successful mtDNA amplification. All hairs exposed to the next highest level of gamma radiation ($1x10^5$), that were extracted for DNA, produced mtDNA amplification products.

Sequences were obtained for all hairs that produced acceptable mtDNA amplification products. These sequences from exposed hairs were identical in type to non-exposed hairs from the same individual. In addition, sequence quality of non-exposed hairs and exposed hairs was comparable, and independent of radiation type.

Collection of hair evidence near or directly from sources of radiation can be hazardous to the individual collector. For example, a gamma dose of 25-40 kGy ($2.5x10^6$-$4x10^6$ rad) is typical for food and pharmaceutical sterilization, and for humans, a whole-body dose of only 0.6-1 Gy (60-100 rad) is fatal almost 100% of the time (the radiation levels in the worst areas of the Chernobyl site are estimated at 200 Gy/hr or $2x10^4$ rad/hr). This study and presentation will give decision makers more data to help determine whether or not the exposure of evidence to certain levels and types of radiation justifies the potential health risk for the evidence to be collected.

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Mitochondrial DNA, Hair, Radiation

A147 A Preliminary Study Into the Characterization and Differentiation of Synthetic Wig Fibers

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After attending this presentation, attendees will learn about the most discriminating features to characterize synthetic wigs when wig fibers are recovered as physical evidence.

This presentation will impact the forensic science community by providing information about chemical and morphological characteristics of wig fibers and their discriminating factors.

Consider a set of fibers that were recovered from the garments of a victim. The analyst will first need to verify if the recovered specimens are hair or wig fibers. If the specimen is from a wig, the analyst has to determine if the specimen has synthetic or natural origin. In having done this, the analyst must then discover identifying characteristics, such as the wig type, and ideally the manufacturer in order to aid in the investigation. The goal of this study is to determine what characteristics of wigs are polymorphs, to develop the most discriminating analytical sequence for microscopic and chemical examinations of wig fibers, and to interpret the value of the results.

Wigs are worn in a variety of settings. Subsequently, there are two main types of wigs: costume and cosmetic. Costume wigs can display a variety of colors and usually have stiff, waxy strands. Cosmetic wigs mimic the appearance of natural hair. The hair used to make wigs can be made of either synthetic fibers or human hair. Nylon, polyester, polypropylene, acrylic, and modacrylic fibers are the most commonly used fibers, with modacrylic being the most prevalent one.

In this preliminary study, a number of samples taken from dark-colored modacrylic, polypropylene, and nylon wigs were collected. These samples of synthetic wig fibers were first visualized under light microscopy using bright field illumination and double polarization. The thickness of each fiber was measured at ten randomly selected positions. Five cross sections were also created from randomly selected positions of each fiber. Various measurements, including, but not limited to, the surface area, circularity, and perimeter, were completed for each of the cross sections. By observing the data distributions through a series of histograms, it was shown that a small amount of wigs had a high degree of intra-variability in both shape and diameter. In some instances, various types and colors of fibers are blended throughout one wig so that it may appear more natural looking for cosmetic use. There was found to be important overlap in the thickness and surface area measurements between wigs. However, it was observed that the cross-sectional shape is a variable feature that can increase the discriminating power of the analytical sequence.

At least 100 different samples of dark-colored synthetic wigs are being collected in the current study. Each sample of synthetic fibers is first examined visually and microscopically. The physical properties of the fibers, such as the color, thickness, and cross-sectional shape are documented. Chemical examinations are conducted by Fourier Transform Infrared (FTIR) spectroscopy and Thin-Layer Chromatography (TLC). The former is used to determine the general polymeric class and subclass when possible (i.e., acrylics). IR data is useful to study the distribution of the fiber types in order to evaluate trends about their rarity of occurrence. The latter method, instead, is used to study the dye content. Dyes are first extracted from the individual wig fibers. This operation allows the obtaining of information about the dye type according to its application mode: acid, basic, or disperse. The consecutive dye elution then informs about the variation of dyes between different wigs. Attempts of dye identification can be made by comparing TLC data of the collected wigs with those of standard dyes. It is important to underline that wig evidence may be recovered as long fibers, about 15cm. This constitutes an advantage because the quantity of evidence will be large enough for applying destructive testing, and in the case of thin layer chromatography, the chances to extract the dye from a single wig fiber are higher.

Wigs, Fibers, Trace Evidence

A148 Using *Pinus* STR Profiling to Discriminate Pollen Samples at the Regional Level: A Potential Tool for Forensic Investigations

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After attending this presentation, attendees will understand the potential to use Short Tandem Repeat (STR) markers to perform DNA profiling of pollen samples from *Pinus echinata*.

This presentation will impact the forensic science community by providing another quantifiable piece of evidence that investigators can utilize to solve crimes and will demonstrate the potential to use short tandem repeat (STR) markers to perform DNA profiling of pollen samples from *Pinus echinata*. The use of a DNA-based STR analysis investigation using primers designed for previously identified STR markers in *P. echinata*, located regionally in southeast Texas for the study of localized population dynamics, may enable discrimination of pollens at regional and local levels. Eventually, this would allow an association between hortleaf pine tree populations or individual pines and associated pine pollen during a forensic investigation.

Advances in plant genomics have had an impact in the field of forensic botany. However, the use of pollen DNA profiling in forensic investigations has not yet been applied. DNA profiling of *P. echinata* pollen using five short tandem repeat (STR) markers is described herein and data supports that there is sufficient genetic variation to discriminate pollen samples at the regional level in southern Texas. Genomic DNA was extracted from pollen and needle samples and then quantified by real time PCR. Species were verified by sequencing the internal transcribed spacer (ITS) regions. DNA was amplified by PCR using fluorescent dyes and the genotype analysis by capillary electrophoresis revealed distinct DNA profiles from trees from 10 selected geographical sites.

This study has demonstrated that a preliminary multiplex STR system, developed from already existing STR markers for *P. taeda* for the study of pine population dynamics, may potentially be used for forensic identification purposes in other pine species. Even though PCR primers were not designed for the particular species of interest, they are sufficiently homologous to enable screening of polymorphic sites as a proof-of-principle for marker evaluation. The use of *P. taeda* primers for the amplification of STRs in the closely related species *P. echinata* was demonstrated to discriminate pollen samples from ten different sites. A DNA extraction method for pollen and a real-time PCR method for accurate pine DNA quantitation were used. In addition, five STR loci from both pollen and needle DNA were successfully typed. DNA profiles obtained from pollen material (*P. echinata*) at ten different sites were perfectly differentiated. When pollen DNA profiles were compared to their conifer donors (reference), no evidence of mixtures was detected for the five amplified STR loci. A population survey indicated the cumulative probability of identity using these five loci could be as low as one in 189. However, a probability to the pollen needle match was not assigned since two out of five markers utilized in this study showed departures from Hardy-Weinberg equilibrium. The causes of Hardy-Weinberg disequilibrium may be due to primer mismatches, small sample size, or the effects of population substructure. Further research is needed to develop a valid statistical approach and a reference database for associations or matches. Despite the departure of markers from Hardy-Weinberg equilibrium, our results strongly suggest that the use of genetic profiling in forensic botany has the potential to offer invaluable evidence in solving crimes where pollen is properly identified, packaged, stored, and analyzed.

Forensic Science, DNA Typing, Forensic Botany

A149 Persistence of Volatile Organic Compounds Associated With Human Decomposition on Carpet Samples

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After attending this presentation, attendees will understand the persistence of chemical residues associated with human decomposition on carpet samples, presenting the "range of detection" for the residues representing putrefied samples, including air flow as a variable.

This presentation will impact the forensic science community by informing attendees of the persistence of volatile compounds associated with human decomposition on carpet samples, giving investigators a better understanding of the presence of select compounds associated with the putrefaction stage of death after a victim's body is removed.

There are few published studies examining the process of human decomposition odor analysis. Recent research suggests that certain chemicals, such as sulfur compounds, are present in early decomposition and can become detected in smaller traces as time progresses. Research is needed to determine the persistence of these Volatile Organic Signatures (VOS) in different environments, especially when the source has been removed. Investigators could determine whether a decomposing body had been removed from a certain location, such as the trunk of a car, by taking an air sample from the location. This study proposes depositing volatile compounds associated with decomposition onto carpet samples, then using SPME to collect and analyze the persistence of these compounds over time.

The volatile compounds chosen for this study were compounds known to be released by human cadavers during the putrefaction stage of decomposition. There are several reasons as to why compounds produced during putrefaction were selected in preference of compounds produced during other stages. Volatile odor compounds cannot be detected during the fresh or autolysis stage. Even though many of the compounds are expected to exist in higher concentrations at the decaying stage rather than the putrefaction stage, the stages of decay and diagenesis can take several months to years to complete. It stands to reason that if a perpetrator had to move the victim's body, the removal would be performed during the first few months since death.

According to the Decompositional Odor Analysis Database, there are 478 separate volatile compounds associated with buried decomposition. Of those 478 compounds, 30 were found to be key substances in human decomposition in soil. Of those 30 substances, 12 are the most significant. They are carbon tetrachloride, toluene, ethane (1,1,2-trichloro-1,2,2-trifluoro), 1,4 dimethyl benzene, benzene, ethyl benzene, decanal, nonanal, hexane, benzenemethanol (alpha-alpha, dimethyl), 1,2 benzenedicarboxylic acid (diethyl ester), and undecane. Cadaverine and putrescine are two notable products associated with human decomposition; however, they have proven difficult to detect due to their low volatility.

This study proposes to adapt previously published headspace analysis method for testing materials that have been exposed to the compounds listed above.[1]

A solution was prepared containing chemicals that might be detected in an enclosed space after a putrefied body has been removed from the area. The chemicals used for analysis were: carbon tetrachloride, toluene, ethane (1,1,2-trichloro-1,2,2-trifluoro), 1,4 dimethyl benzene, benzene, ethyl benzene, decanal, nonanal, hexane, benzenemethanol (alpha-alpha, dimethyl), 1,2 benzenedicarboxylic acid (diethyl ester), and undecanal. The exact quantities of each compound in this solution will be determined following initial experiments detecting the compounds individually.

A total of 20 (2 in. x 2 in.) olefin carpet samples were placed in 20 separate Erlenmeyer flasks. An aliquot of 1mL of the chemically synthesized putrefied sample was deposited onto each carpet substrate. Air samples were taken over an eight-week period.

Ten flasks were sealed with silicone to prevent air from interfering with the samples, and placed in a dark storage area. Ten flasks

remained unsealed in a chemical hood. Each week, a sample from both conditions was collected using headspace-SPME. Once removed from the hood, the unsealed flask was sealed to allow for the headspace sample collection.

The extraction method involved the placement of sorbent material (SPME fiber) into the headspace of the two flask samples. A solution containing either chlorobenzene or bromobenzene was used as an internal standard. For this study, the exposure time for each sorbent material began at 30 minutes. The SPME samples were analyzed using gas chromatography/mass spectrometry.

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Volatile Compounds, Decomposition, SPME

A150 Development and Validation of a PCR Amplification Kit: An STR Multiplex System for Teaching and Research Purposes

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After attending this presentation, attendees will have a basic understanding of the methodologies and techniques needed to create a 4-loci Short Tandem Repeat (STR) multiplex kit for teaching and research purposes.

This presentation will impact the forensic science community by providing the groundwork that teaching and research facilities need to create a cost-effective STR kit. By lowering the cost of each STR reaction, teaching facilities can perform more reactions and therefore, provide their students with more opportunities to improve their techniques in a DNA laboratory with the ability to determine the success of research projects at a fraction of the cost.

Although numerous commercial kits have been developed to amplify STR loci, commercial kits are often expensive. Since these kits are used for discrimination purposes, 16-loci provide the statistical confidence needed for testimony. Since these loci are spread throughout the genome, every allele is inherited independently and the markers are not linked. In this way, a complete profile has powerful discriminatory capability as the probability of individuals with the identical alleles decreases as more loci are analyzed.

Not all facilities that use these kits, require such power of discrimination, resulting in wasted resources. Teachers can demonstrate multiplex STR amplification and analysis using reactions that amplify fewer loci. The purpose of most forensic biology laboratory exercises is to teach the basics of PCR amplification and provide students with hands-on experience setting up and performing the reactions. Although these exercises may also include examining and comparing allelic profiles, making an identification with a high statistical level of confidence is not necessarily an objective. Therefore, using the commercial kits to perform classroom laboratory experiments could be considered wasteful and unnecessarily expensive. A kit that amplifies at least three loci using two dyes would be useful for classes conducting multiplex reactions.

From a research standpoint, a simple kit could be used to screen samples and predict the success of a commercial kit. For forensic DNA research projects, often the objective is to determine whether amplifiable DNA is present in a given sample. The results from less expensive monoplex and multiplex reactions would provide data about whether amplifiable DNA exists at chosen STR loci. A less expensive kit would allow investigators with a limited budget to process more sets of samples and include more samples in a given set. Often, evidence samples, or mock evidence samples, will contain small amounts of DNA (fingerprint residues) or be exposed to harsh environments (fired cartridges). These conditions can result in variation of results within a data set, where some samples may amplify all STR loci included in the reaction, while other samples will not contain any amplifiable DNA. Including more samples in each data set will help investigators better evaluate the variability of results within a given set of samples.

The goal of this project was to develop and validate a more cost-effective STR amplification kit specifically designed for research, screening, and teaching purposes. For this study, 4 STR loci were chosen for amplification, D3S1358, Th01, D8S1179, and vWA. Dye-labeled primers were designed for each loci based on previously published sequence data. A ladder was developed for analysis. Monoplex reactions were performed in order to evaluate the best amplification conditions for each locus. Once the multiplex reaction was balanced to produce even amplification among the 4-loci, a validation study was performed, evaluating thermal cycling parameters, precision, and sensitivity.

STR Multiplex, Teaching Kit, DNA

A151 Preparing Next Generation Sequencing (NGS) Libraries of Human Mitochondrial DNA Using Illumina® Nextera® XT and NEBNext® dsDNA Fragmentase® Technology

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After this presentation, attendees will have gained insight into the differences between commercially available enzymatic DNA library preparation methods for next generation sequencing, focusing on practical applications of these methods to human mitochondrial DNA sequencing in a forensic context.

This presentation will impact the forensic science community by making recommendations in regards to best practices for NGS library preparation of mitochondrial DNA, specific sample throughput optimization using multiplexing strategies, and chemistry dependent sequencing artifacts commonly encountered.

Forensic DNA casework largely relies on the analysis of short tandem repeats (STRs) from nuclear DNA (nDNA). In some cases; however, nDNA may not be suitable for analysis (i.e., highly degraded DNA or DNA present in quantities too low to obtain an STR profile). In these instances, mitochondrial DNA (mtDNA) is an excellent alternative. MtDNA is a circular genome of approximately 16.5kb, is maternally derived, and is present in 500-1,000 copies per cell versus two copies of nuclear DNA. The combined higher copy number, circular shape of the genome, and location in the mitochondria allow for a greater probability to recover sufficient mtDNA for typing of degraded samples.

Currently, forensic analysts sequence two or three hypervariable (HV) regions found in the non-coding control region of the mtGenome since sequencing of the entire genome is rather labor-intensive. Additionally, sequencing difficulties of the C-stretch and the identification of heteroplasmy in samples can add complexity to the analysis of mtDNA evidence in casework when traditional Sanger sequencing methods are used. These issues can be addressed by introducing next generation sequencing (NGS) technologies to the crime laboratory. NGS is a high-throughput technique that combines hundreds of thousands of sequencing reactions simultaneously, and allows for the sequencing of whole genomes more rapidly. Furthermore, NGS enables deeper analysis of the genome for identification of minor variants since many reads of a single template sequence are obtained.

Library preparation is the primary bottleneck in the NGS workflow, since it can be very time consuming. Therefore, the goal of this research is to compare two enzymatic NGS library preparation methods, Illumina® Nextera® XT and New England Biolabs NEBNext® dsDNA Fragmentase®. The Illumina® Nextera® XT kit is designed exclusively for use with Illumina® instrumentation. This kit uses an engineered Transposome™ to randomly fragment and tag small amplicons with

Illumina® specific adapters. The NEBNext® dsDNA Fragmentase® kit is designed for use with all major NGS platforms, and employs a family of enzymes to nick dsDNA randomly, ultimately allowing for platform specific adapter ligation.

For this study, DNA was extracted from buccal swabs obtained from eight donors according to approved IRB protocol. Long PCR using a highly processive polymerase and novel primer sets designed by the authors was successfully performed on these extracts to independently amplify the mtgenome in two amplicons overlapping at the control region. Additionally, whole genome amplification (WGA) was performed on a series of dilutions of the buccal extracts, to mimic DNA concentrations encountered with compromised samples.

Following mtDNA amplification, samples were treated with either Illumina® Nextera® XT or New England Biolabs NEBNext® dsDNA Fragmentase®. Preliminary data has shown these library preparation methods to be successful with long PCR amplicons derived from pristine DNA, suggesting a streamlined library preparation method for use in databasing laboratories. Next generation sequencing data for these samples has been generated using both the Roche 454® GS Junior and the Illumina® MiSeq® platforms. Each NGS platform and library preparation method gives rise to sequence differences between the samples. More research is currently being conducted in our laboratory to further elucidate causes of these differences.

Next-Gen Sequencing, Library Preparation, Mitochondrial DNA

A152 The Evaluation of Six Different Matrices for the Collection of Touch DNA

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After attending this presentation, attendees will gain an understanding of the ability for various matrices to collect touch DNA from a variety of surfaces.

This presentation will impact the forensic science community by demonstrating that the DNA yield, determined by RT-PCR quantification, and STR profile quality, determined by capillary electrophoresis, can be significantly impacted by the swab matrix and collection method selected.

Touch DNA samples from crime scenes are frequently collected by law enforcement agencies around the world. These samples inherently contain less genetic material as compared to other biological stains (i.e., blood and seminal fluid). The ability of one swab matrix to collect even a few cells more than another could have a substantial impact on the generation of a complete STR profile.

The collection of genetic material from a surface is only a subset of the overall process. Collecting significantly more genetic material from a surface is beneficial only if the cells are subsequently released from the collection matrix, lysed during the extraction procedure, and purified for downstream applications. The size, shape, composition, and hydrophilic/hydrophobic properties of each matrix play an integral role in a swab's ability to collect and successfully release a touch DNA sample for DNA purification.

This study tested six (n=6) different matrices for Touch DNA evidence collection which were cotton, foam, rayon, knitted polyester, woven polyester, and glass fiber.

Similar to the types of samples that may be encountered during routine evidence collection, a variety of surfaces were evaluated during this study. Flat surfaces such as vinyl and composite wood paneling tiles were evaluated. Curved or fabric surfaces such as a polyvinyl chloride (PVC), knitted gloves, and polypropylene rope were also selected for evaluation. Aside from testing various surfaces, multiple other variables were also studied to determine the impact, if any, on each swab matrix's ability to collect a sample. These variables included multiple methods of swabbing (wet vs. dry collection), different wetting agents (DNA Grade Water vs. Isopropyl Alcohol), the number of swabs used for collection (one vs. two), and the analyst collecting the sample.

During this study, volunteers (n=2-5 depending on the surface) were asked to apply either fingerprints or handprints, handle objects ,or wear gloves at various time intervals over the course of several days. After all samples were deposited, the surfaces were collected and swabbed using each of the six matrices.

Experimental design of this study was controlled as much as possible. Surfaces were cleaned and stored in a controlled area prior to and after sample deposition to limit the possibility of exogenous DNA. Volunteers deposited samples on surfaces within specific dimensions to control the area of sample collection. In addition, control swabs were utilized throughout the study to assess the difference in genetic material deposited on each surface by either the right or left hand.

Data analysis and comparison were aided by the statistical calculations computed using the JMP® Design of Experiments software. The statistical calculations focused on DNA yield and the resulting STR profile with normal amplification parameters.

The results from this study will demonstrate that the difference in DNA yield from samples deposited by the right or left hand were not statistically significant, at a given time point, allowing for paired sample analysis. This study will also demonstrate that statistically significant differences in DNA yield can be obtained through the use of different swab matrices. The resulting differences in DNA yield subsequently affect the ability to obtain a complete STR profile.

Woven polyester, glass fiber, and knitted polyester displayed statistically significant differences compared to the other swab matrices tested in terms of yield or STR profile. Depending on the surface to be sampled, wetting agent utilized, and method of collection, an analyst may find it advantageous to explore nonstandard swab matrices in order to achieve a higher yield of DNA and a more complete genetic profile.

Touch DNA, Swab Matrices, Collection Technique

A153 Metrological Issues Concerning the Analysis of Brazilian Heavy-Metal Free GSR by SEM

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After attending this presentation, attendees will understand issues regarding the size and composition of Gunshot Residue (GSR) particles that may affect and even impair an accurate result.

This presentation will impact the forensic science community by discussing unique metrological issues that are usually overlooked during GSR analysis, recognizing the differences from the pressure to produce environmental ammunitions may impair the accurate identification of GSR by all known methodologies.

Metrology is defined as the science that includes all theoretical and practical aspects of measurement. Although not disseminated among the forensic science and justice communities, it is of utter importance since accurate measurement results can have an impact on the outcome of a trial. Most forensic analysis relies on qualitative techniques, which probably explains why metrology is so overlooked. Yet even qualitative analysis can benefit from metrology. One such example is GSR particle analysis. GSR is probably the most common and at the same time underestimated type of trace evidence, despite the growing number of firearms-related offences in Brazil. GSR particles are generated by a combination of high temperature and high pressure conditions occurring during firearm discharge. Particles are mainly composed of elements from primer mixture (mostly heavy metals) and so are very stable. They are also non-crystalline and circular in shape. The technique of choice is SEM-EDX and results are expressed in qualitative terms: presence or absence of particle types as defined by the ASTM E-1588/10e1 standard. However, the detection of particles and its identification by automated SEM systems is greatly dependent on metrology. For instance, identification is based on particle diameter range and element composition. In this study, metrological aspects of GSR detection were analyzed by SEM. Automated GSR particle

analyzer software base their identification on three parameters: BSE brightness given by Z contrast, circularity factor, and element composition. These parameters rely on the ability of the instrument (i.e., the SEM and the EDX) to measure with accuracy. This implies that the BSE detector and X-ray probe must be adjusted and magnification calibrated.

Validation of the method was performed employing Ted Pella SPS-5P-2 GSR Certified Standard, ENFSI synthetic GSR standard, and an in-house GSR-like sample containing LaCe(Fe) particles (0.5-20µm). In the case of this research instrument, an FEI Quanta 200 ESEM equipped with a Genesis EDAX EDX and GSR-XT software, the instrument detection limit (IDL = 0,8) was calculated in regard to the smallest particle size detected, employing the CRMs, meaning that smaller particles were not detected, except in manual search. The sub-micron detection capability of laboratories from four different proficiency tests using a similar RM were reviewed and found that approximately 50% were considered unsatisfactory for 0.5µm particles. This percentage is steady from 2001 to 2008. These laboratories are recommended to disregard sub-micron GSR during real examinations. Yet, the inability to detect sub-micron particles is critical for Brazilian samples, due to the tendency of CBC primer to generate such smaller particles. Accuracy was tested applying the Z-Score to the results of seven runs and was considered unsatisfactory (the mean z was -9,98, meaning that results were almost 10x below the correct mean number) when the software was installed. At this time, on average 90% of the 2.4µm particles were detected. Satisfactory mean Z-scores were achieved after calibration of magnification using a CRM and of X-ray probe adjust employing a metal CRM, resulting in the detection on average of 90% of all 0.8µm particles. Although a rather acceptable reproducibility was obtained (same sample and parameters over different days and operators), repeatability (same sample and parameters over a short period of time) was not acceptable (*s.d.* = ± 3) against a preferred 1.3 for 0.5µm particles. A great concern resulting from the analysis of Brazilian ammunition generated-GSR is the size of particles. GSR range between 0.5 and 20µm in diameter, but particles generated by CBC ammunition are mostly sub-micron. On one hand, this may increase its potential as a hazardous airborne particle, although it also imposes some difficulties in detection by most SEM systems because of: (1) pixel limitations; and, (2) electron transparency. A minimum 20kV accelerating voltage is required to detect PbL lines, but this may be a problem for sub-µm particles because of the interaction volume and reduced number of BSE. Most systems detect particles >0.5µm, which in reality corresponds to 0.7µm particles. The characteristics arising from CBC-generated GSR probably mean that a smaller beam diameter is needed and associated to a rigorous control of SEM parameters to ensure accurate results.

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Gunshot Residues, Microscopy, Metrology

A154 Analysis of Fatty Acid Amide Hydrolase (FAAH) Inhibitors in the URB Series in Botanical Materials by GC/MS and LC/MS

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After attending this presentation, attendees will be able to evaluate methods for analysis of Fatty Acid Amide Hydrolase (FAAH) inhibitors in the URB series, in botanical materials using Gas Chromatography/Mass Spectrometry (GC/MS), and Liquid Chromatography/Mass Spectrometry (LC/MS).

This presentation will impact the forensic science community by describing a screening process to identify a novel class of synthetic drugs with effects on the endocannabinoid system in botanical materials.

FAAH's compounds inhibit the enzyme Fatty Acid Amide Hydrolase (FAAH), which is believed to be responsible for, among other things, the degradation of the endogenous cannabinoid receptor ligand, anandamide. Anandamide is linked to pathways responsible for mediating anxiolytic, antidepressant, and analgesic effects. In particular, a series of FAAH inhibitors developed at the University of Urbino in Italy, and designated URB, followed by a series number, have been detected in "legal high" products being sold over the Internet. Although these compounds don't act directly on the cannabinoid receptor, they produce similar effects to marijuana by increasing the levels of the endogenous levels of anandamide. In theory, this may result in cannabis-like effects including euphoria and intoxication.

Five compounds from this series, URB-597, URB-602, URB-447, URB-754, and URB-937 were selected for study. The neutral character of URB 602, URB 597, and URB 937 is due to the presence of the carbamate group in their structure, which tends to make them subject to hydrolysis and consequently unstable.

Standards were prepared in methanol and analyzed by GC/MS. Gas Chromatography conditions included the use of a 15m Phenomenex Zebron ZB-5MS, 0.25mm ID column, with a temperature program from 150°C to 325°C at 18 degrees/minute.

URB-602, URB-447, and URB-754 were determined to be stable under these conditions. URB-602 produced major ions at m/z 195, 169, 213, and molecular ion of 295, while URB-447 produced fragments at m/z 400 (molecular ion), 275, 105, and 125. URB-754 had masses as follows; 160, 266 (molecular ion), 104, and 77.

URB-597 and URB-937 showed the presence of degradation products by GC. The peak corresponding to URB-597 showed fragments at m/z 213, 197, 169, and 139, while URB-937 gave fragments with m/z 212, 184, 229, and 128. These degradation products most likely represent hydrolysis of the molecule at its carbamate bridge. URB-597 shares components of its structure with another FAAH inhibitor, JP-104.

Standards were also analyzed by single quadrupole LC/MS. The mobile phase used was 65% Acetonitrile: Isopropanol and 35% 0.1 TFA in water, analyzed on an Eclipse plus 4.6x100mm C-18 column (Agilent). Chromatography was consistent by LC/MS with no evidence of degradation or breakdown.

Extraction procedures were evaluated for URB-602, URB-447, and URB-754. Acid, neutral, and basic extraction procedures were evaluated using pH 7.4 potassium phosphate buffer and 90/10 methylene chloride/ isopropanol. GC data from the analysis of several "legal high" products indicated the presence of URB 754 in products including Kush Hypnotic 20XXX, White Widow 5X, B2 Da Bomb Blueberry Potpourri, Conviction 20XXX, and Dank 15X botanical materials. URB-754 was present in each case with a synthetic cannabinoid compound including AM-2201, JWH-022, and JWH-018 Chloropentyl analog.

URB Compounds, FAAH Inhibitors, Botanical Material

A155 Interpretation Challenges Using the PowerPlex® 16 HS Kit for Forensic Casework

Amy M. Jeanguenat, MFS, 10430 Furnace Rd, Ste 107, Lorton, VA 22079*

The goal of this presentation is to educate the forensic DNA community on interpretation challenges faced, especially in mixtures, when amplifying forensic casework samples with PowerPlex® 16 HS.

This presentation will impact the forensic science community by increasing the knowledge of additional common artifacts and anomalies seen in casework samples amplified with PowerPlex® 16 HS that may not be noted in technical manuals or validation studies, which will help during interpretation, comparisons, and reporting.

With the evolution of next generation amplification kits, manufacturers are competing to deliver products that meet the demands of the forensic DNA community. This includes overcoming inhibition, increasing sensitivity, adding discrimination power, and reducing overall amplification time. Although amplification kits undergo developmental

and internal validations, once applied to actual unknown samples they may not behave as predicted. Bode internally validated PowerPlex® 16 HS in a 25µl reaction with 30 total cycles and found it to be reliable, accurate, reproducible, and precise. An examination of mixtures, contamination, and artifact assessment was also performed. During validation, this kit outperformed other amplification kits in its efficiency to overcome inhibition. However, optimization of reagents to increase robustness in the PowerPlex® 16 HS amplification kit has led to an increase in amplification artifacts and anomalies that can make interpretation challenging.

An assessment of artifacts during validation supported an increase in n-4 stutter over the manufacturer's recommendations at D3S1358 (increase from 11.22% to 13.7%) and CSF1PO (increase from 8.15% to 11%). In general, excessive stutter occurred more frequently when amplification reactions generated profiles with alleles greater than 3000 RFU or when n-4 and n+4 peaks were acting on the same stutter artifact. With the exception of the documented dye artifact occurring at 172 base pairs (bp) in the ILS 600, no other dye artifacts were noted.

Once the kit was implemented for use on casework samples, previously unidentified artifacts/anomalies were noted in many samples. This includes a dye artifact at Penta D which ranges from 450bp-500bp and varies in total relative florescent units (RFU) from 300 to 1000 RFU. Other loci displayed uncommon non-specific artifacts including peaks in the n-20 position at TPOX, the n-12 position at TH01, and the n-17 position at Penta D. Occasionally, these peaks fall into sizing bins and can cause confusion in mixture interpretation; however, the most challenging new artifact is an increase in possible bacterial peaks. The addition of a third amplicon of equal or greater height (in RFU) than other alleles present at the same location has been noted in several different casework samples at the following loci: TH01, D18S51, D5S818, D7S820, D16S539, and CSF1PO. These artifacts occurred in sexual assault samples processed within the same year they were collected. The sample types ranged from rectal swabs, tampons, outer labia swabs, peritoneum swabs, and oral swabs. These additional peaks are assumed to be non-human in nature and can be rectified by confirming data with other amplification kits. Finally, discordance between samples has also been noted at Penta E between PowerPlex® 16, PowerPlex® 16 HS, and/or PowerPlex® 18D. Collections of these artifacts led to an internal investigation and discussion ultimately resulting in several interpretation changes needed to effectively interpret samples amplified with this kit.

Case examples, displays of artifacts/anomalies, and changes to interpretation guidelines to accommodate casework processing will be provided.

PowerPlex® 16 HS, Artifacts, Interpretation

A156 The Characterization of Bullet Wipe From Non-Lead Bullets Using Laser Induced Breakdown Spectroscopy

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After attending this presentation, attendees will have understanding of how laser-induced breakdown spectroscopy (LIBS) can be used for the presumptive identification of trace evidence, learning how this technology also can be used to detect bullet wipe from non-lead bullets.

This presentation will impact the forensic science community by presenting a new method for the presumptive identification of bullet wipe in bullet holes shot from a distance of over six feet; a distance that gun powder residue is unlikely to travel. Over the years, scientists have developed methods for detecting GSR on a shooter's hands, a victim, or any object that may have been within six feet of the firearm. However, when the weapon is more than six feet from the target, gun powder residue is generally not present. The only residue available for analysis is bullet wipe, material that is transferred from the surface of the bullet as it penetrates the target.

Laser Induced Breakdown Spectroscopy (LIBS) is used to identify the elemental composition of an unknown material. Areas as small as $2mm^2$ can be analyzed, making it an ideal method for the analysis of the minute residues left by a bullet. The advantages of LIBS include minimal sample preparation and short time for analysis. This technique is also relatively non-destructive to most surfaces being tested. Unlike atomic absorption, the composition can be unknown. The primary disadvantage of LIBS is that quantification is not currently possible.

The focus of this project is to apply LIBS as a presumptive test where identifying the composition of the sample is sufficient and quantification can be done as part of the confirmatory analysis, if required. The ease of analysis with LIBS lends itself very well to fast screening of inorganic trace evidence.

Four materials, cotton, wood, drywall, and cement, were shot with three different brands of lead-free cartridges: Extreme Shock: 357 Mag 90 CT2 (ES), Dynamic Research Technologies 9mm (DRT), and Winchester Super Clean NT (WIN). Each material was shot from distances of one, three, six, and twelve-feet. To detect the bullet wipe, the samples were analyzed for copper, lead, and barium. The analysis was performed using addLIBS software and the NIST Atomic Spectra Database.

The manufacturers' information stated that the ES bullets were made of copper-clad tungsten, the DRT bullets had a copper-clad compressed core made from three undisclosed metals, and the WIN had a copper clad tin core. Due to the jacket, copper was the only metal detected in the bullet wipe that could be attributed to the cartridges. Some lead and barium was detected in the bullet wipe, most likely from the barrel of the guns, as these metals were not detected in the casing, bullet, or powder from any of the unfired ammunition used for the controls. Lead and barium were found on the swabs used to clean the barrel of the gun between firing.

Bullet wipe was successfully detected in the cotton, wood, and drywall. Bullet wipe could not be detected in the cement block. Not only did the bullets knock chips off the surface of the block, but cement is a complicated mixture of minerals, including barium oxide.

Gun Powder Residue, LIBS, Bullet Wipe

A157 Compound-Specific Carbon and Hydrogen Isotope Ratios of Paraffin for Forensic Investigations

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After attending this presentation, attendees will appreciate the forensic isotopic information contained in paraffin and hydrocarbon component mixtures.

This presentation will impact the forensic science community by providing additional discriminating isotopic tools for in-depth paraffin and hydrocarbon investigations.

Paraffins and, in general, hydrocarbon mixtures have a high significance in forensic investigations. Paraffin products are used (e.g., for candles), to impregnate matches or in cosmetics. Hydrocarbons in general are also used as arson accelerants. Remnants of liquid products such as paraffin, petrol, or diesel can often be identified at the crime scene and sometimes on ignition devices, for example, safety matches or candles.

Presently, paraffins and other (volatile) hydrocarbon mixtures are mostly characterized using GC or GC/MS methods. Some successful research has also been done on characterization of paraffin-containing candle waxes as part of an extended arson investigation.[1]

Further discrimination can; however, be obtained by using ^{13}C and ^{2}H isotope information for each single paraffin or hydrocarbon component in the mixture. This will provide a whole new dimension to the characterization of these materials. A GC-IRMS/MS method was developed and validated. Validation results will be shown. The GC-IRMS/MS method was then applied to characterize candle waxes,

paraffin products as used to impregnate safety matches, as well as hydrocarbon mixtures as used for lamp oils.

The additional discrimination of GC-IRMS/MS over the already strong discrimination provided by EA-IRMS will be demonstrated for candle waxes from candles of various brands and shops from the Netherlands that were previously investigated using gas chromatography in combination with bulk carbon and hydrogen isotope ratios.

For the second application, paraffin was extracted from wooden safety matches in the NFI collection of wooden safety matches with burnt and un-burnt samples. Initial compound-specific n-alkane ^{13}C and ^{2}H isotope data from 14 wooden sticks of six different brands of safety matches indicate a wide variation in isotope values from -10‰ to -80‰ VSMOW for hydrogen and -29‰ to -35‰ PDB for carbon. In addition, the effect of burning on the isotope ratios is shown to be negligible for these samples. Further statistical data evaluation using the technique of cluster analysis will be performed to evaluate the potential level of discrimination such as between and within packages of safety matches from one brand.

For the last GC-IRMS/MS application, nine lamp oils from the NFI lamp oils collection were used, nominally representing five different brands. Both for ^{13}C as well as for ^{2}H isotopes, a wide variation is observed. Single brand lamp oils as bought in various shops could easily be discriminated in this way.

The above investigations are only a first step in applying GC-IRMS/MS to hydrocarbon mixtures in a forensic context. For some applications (candles, cosmetics), method application appears to be straightforward. For other applications, further investigations will be required. For example, in arson investigations, remnants of hydrocarbon mixtures as used for accelerants will normally be strongly evaporated, skewing the isotope ratios of the components. Furthermore, pyrolysis products from other materials may be present in a fire residue sample, potentially resulting in complex chromatograms where the hydrocarbon components to be investigated cannot be separated from other components.

Reference:

1. Dogger J, van Breukelen M, Hendrikse JN, Schrader MA, van Grol M, van der Peijl GJQ. Discrimination of candle wax materials by gas chromatography (GC) and isotope ratio mass spectrometry (IRMS). *Proceedings of the American Academy of Forensic Sciences*, 61st Annual Meeting, Denver, February 17-21, 2009, 103-104.

GC-IRMS, Paraffin, Isotope

A158 Identification of an Impurity in Methamphetamine Synthesized Via Reductive Amination

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After attending this presentation, attendees will better understand the importance of classifying methamphetamine based on synthetic route and one specific way in which that task is accomplished. Classifying methamphetamine based on synthetic route provides valuable intelligence concerning current manufacturing trends, which in turn leads to the effective monitoring of precursor and essential chemicals. Even in high-purity products, trace amounts of such route-specific impurities remain. Since manufacturing methods are ever-changing, it is imperative to keep abreast of these key "marker" impurities by isolating and identifying them, and determining their significance in any given sample.

This presentation will impact the forensic science community by making synthetic route identification of methamphetamine a little easier, therefore enhancing the communitys ability to track synthesis trends and movement of the drug.

In this study, one previously unidentified impurity was targeted for isolation and structural elucidation because of its presence in roughly 26% of seized methamphetamine samples produced via reductive amination that were analyzed at the DEA Special Testing and Research Laboratory in the past year. The samples were seized from various locations throughout the United States and port of entry locations at the Mexican border. A majority of the methamphetamine seized in the United States has been smuggled from Mexico where it is synthesized using one of two common reductive amination methods: the Mercury-Amalgam method and the Leuckart method. Both methods use phenyl-2-propanone (P2P) as the precursor, but subsequently use different essential chemicals. Very few reliable impurities have been identified that are specific to the Mercury-Amalgam (also known as the "Biker Method") versus the Leuckart methods, making classification difficult. The targeted impurity in this study is theorized to be synthesized by the Leuckart method.

Isolation and elucidation were carried out using a methamphetamine hydrochloride exhibit that contained approximately 1% of the target impurity. The sample was extracted in a phosphate buffer designed to remove most of the methamphetamine from the impurity. Preparative high performance liquid chromatography was then performed on the extract to isolate the target compound from the remaining methamphetamine and any other minor impurities still present. Once completely isolated, carbon and proton nuclear magnetic resonance spectroscopy were performed to elucidate the structure of the impurity. With the structure known, the mechanism of its formation can be identified, and its value as a route-specific marker compound established.

In regards to the forensic community, this study will improve the classification of the synthetic route used to manufacture the methamphetamine, ultimately aiding the intelligence community with the monitoring of methamphetamine manufacturing trends as well as the trafficking of the drug and its precursors. The identification of another potential route-specific marker adds an additional distinguishing factor between exhibits. Since methamphetamine is completely synthetic, the place of manufacture cannot be determined by simply looking at the chemical analysis of the exhibit. Instead, methamphetamine exhibits are analyzed, classified by synthesis route, and then compared to one another by looking at several characteristics identified during analysis, especially impurities left behind from synthesis. The results of these comparisons are used to update methamphetamine manufacturing trends which in turn support the intelligence and law enforcement communities with the monitoring of precursors and essential chemicals.

Methamphetamine, Synthesis, Impurity

A159 Multivariate Empirical Bayes Methods Applied to the Identification of Impression Evidence

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After attending this presentation, attendees will better understand how to apply empirical Bayes multivariate statistical methods to the identification of impression evidence, specifically firearm and tool markcomparisons.

This presentation will impact the forensic science community by presenting examples and explanations of empirical Bayes methods for the identification of impression evidence.

For several decades, and especially since the National Academy of Sciences' 2009 Report *Strengthening Forensic Science in the United States*: *A Path Forward*, forensic firearm and tool mark comparisons have been under increased scrutiny. A significant criticism of the analysis of pattern and impression evidence is that there is no accepted

methodology to generate numerical proof that independently corroborates morphological conclusions. This research critically evaluates the use of empirical Bayes methods for the analysis of firearm and tool mark impression evidence.

For more than half a century, empirical Bayes methods have been applied to many types of problems in a plethora of disciplines including, but not limited to, genomics, economics, epidemiology, safety engineering, and quality assurance. Its rise in popularity can be attributed to the increase in data set sizes, computing power, and because of the straightforward nature of inference based on Bayes' rule. However, empirical Bayes methods have not yet been applied to the statistical analysis of forensic evidence.

The appeal of empirical Bayes methods is that it blends objective frequentist and subjective Bayesian approaches which lead to a falsifiable inference method consistent with the Popperian philosophy of science. Empirical Bayes methods can be interpreted as an approximation to a "fully Bayesian treatment" of a hierarchical model in which the parameters at the highest level of the hierarchy are set to their most likely values, rather than being integrated out. From a forensic science perspective, the most significant advantage of empirical Bayes methods is their ability to formulate clear statistical inferences in which the prior probability is estimated objectively from the data rather than subjectively before the data is observed.

In this research, 3D quantitative surface topographies of firearm and tool markstriated impressions were collected using confocal microscopy. A reasonably complete striation pattern was then summarized as multivariate feature vectors in the form of mean profiles. Next, principal component analysis (PCA) was used to reduce correlation within each profile while maintaining the essential information and a support vector machine (SVM) learning algorithm was used for identification. Last, empirical Bayes methods were used to estimate local false discovery rates (FDR), also known as posterior error probabilities, which quantitatively assesses whether an identification *really is correct*. In order to alleviate the concerns of data reuse, common in empirical Bayes based schemes, we adopted the standard multivariate model fitting practice of first splitting the data into separate training, validation and test sets. The training set was used to generate estimates of a null likelihood and null prior. The validation set was then used to find FDR estimates based on the training set null estimates. This approach has the added advantage that estimates of standard errors for fdrs are available. At each step in the process, goodness-of-fit diagnostics were applied and independence assumptions on the fit *z*-values (required for the standard error formulas) were tested. The FDR estimates can ultimately be used to accompany each identification of an unknown with an output by a machine learning algorithm, providing statistical support for impression evidence conclusions.

Empirical Bayes, Impression Evidence, Statistics

A160 Whole Mitochondrial Genome Sequencing Using Probe Capture and 454 Sequencing Technology

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After attending this presentation, attendees will gain deeper knowledge of sequence capture for enriching mitochondrial DNA and next generation sequencing using 454.

This presentation will impact the forensic science community by introducing a novel capture method for sequencing the entire mitochondrial genome of degraded or limiting samples.

Mitochondrial DNA (mtDNA) analysis is most useful in forensic cases when samples are degraded and nuclear STR testing cannot produce a complete discriminating profile. The most widely used approach for mtDNA analysis is sequencing the hypervariable regions (HVI/HVII) by Sanger sequencing. The maternal inheritance pattern of mtDNA and sequencing information from only the HV regions provides limited discrimination power, particularly in the Caucasian population due to a few common types. Sanger sequencing is also limiting in that it often fails to detect and resolve low level mixtures, as well as low level heteroplasmy; both common observations in forensic mitochondrial samples. The development of a method for whole mitochondrial genome sequencing using a liquid phase hybridization probe capture strategy followed by sequencing using 454 Next Generation Sequencing (NGS) to overcome these limitations will be described. The hybridization probe capture technique allows the entire mitochondrial sequence to be captured for analysis regardless of fragment size by exploiting a very large number of capture probes designed directly from the mitochondrial genome. Utilizing 454 NGS after capture yields tens to hundreds of thousands of sequence reads making it possible to detect mixtures, including heteroplasmy at lower levels, than current Sanger sequencing methods. Moreover, highly degraded DNA samples already consist of small DNA fragments and can be directly subjected to the capture and sequencing analysis.

The Nimblegen SeqCap EZ platform was chosen as the capture platform due to its extensive tiling design and ability to efficiently incorporate hundreds of thousands of capture probes. To increase the specificity of the probes, the circular nature of mtDNA, and the high density and distribution of polymorphisms was considered in the design strategy. The probes were also designed to exclude the capture of known nuclear pseudogenes. The final design directly targets 99.99% of the mitochondrial genome with unique probes.

Results show that 100% of the mitochondrial genome of all samples was captured with coverage adequate to yield unambiguous sequence assignments with an average on target capture rate of 75%. All SNPs previously detected by Sanger sequencing were also detected by 454 sequencing in all samples. Multiple samples have been tested to evaluate the specificity of the assay. The sensitivity of the method was tested by reducing the starting amount of DNA to forensically relevant DNA levels (<1ng sample DNA) with no loss in sequencing accuracy. The method also achieved resolution of mixtures below the limits of Sanger sequencing (<10%). To improve efficiency, the probe capture hybridization time was reduced from the manufacturer's recommendation of three days to one day. This greatly improves the throughput of the capture method, without affecting the on target capture rate, or accuracy of the capture probes.

Current studies focus on improving the sensitivity and robustness of the method. To analyze samples from more diverse populations and then investigate forensically relevant samples is propsed in this study. These samples include ones with even more limited total DNA, and samples which have degraded beyond the limit of conventional STR analysis. In conclusion, a method for whole mitochondrial genome capture followed by NGS which can be applied to the field of forensic science has been successfully developed.

Mitochondrial DNA, 454, Sequence Capture

A161 Mitochondrial DNA Recovery and Analysis From Spent Cartridge Casings

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After attending this presentation, attendees will learn about the utility of mitochondrial DNA (mtDNA) analysis of epithelial cells from spent cartridge casings and the optimal DNA recovery method from such evidence.

This presentation will impact the forensic science community by detailing a highly sensitive DNA analysis method that allows for the identification of a weapon's handler using mtDNA recovered from spent cartridge casings.

It is not uncommon that the only evidence remaining at the scene of a shooting is spent cartridge casings. Because cartridges are usually loaded into a firearm by hand, forensic scientists have long attempted

obtaining fingerprints from spent cartridge casings, but these efforts are almost never successful. An alternative method for identifying the person who loaded bullets into a clip or chamber of a firearm comes from the epithelial cells that may be deposited onto handled cartridges. Previously, scientists have examined the feasibility of STR typing of DNA from spent cartridge casings, but have had limited success, most likely due to a combination of low copy number DNA, DNA degradation caused by temperatures reaching 1800°C during firing, and PCR inhibition resulting from the metal and/or primers in the gunshot residue.

While obtaining even a partial STR profile from spent cartridge casings can be beneficial, in most cases not enough DNA data is recovered to link a suspect to the scene. In this regard, there is a greater likelihood of obtaining DNA typing results by focusing on mtDNA due to its higher copy number and protection in the mitochondrion. In spite of this, however, researchers have not looked into the inherently promising mtDNA analysis as a tool for identifying the handler of cartridges.

In this study, volunteers loaded ten 40-caliber cartridges each into the magazine of a gun. The cartridges were then fired and DNA was recovered from five of the ten fired casings individually using a double swab method—a wet swab followed by a dry swab—for each casing. For the remaining five casings, one pair of swabs was used to swab them all, resulting in a "cumulative swab." A standard organic extraction, followed by the use of spin columns for removal of PCR inhibitors, was used to purify the DNA. Yields were determined, and no statistical difference between the recovery methods was found.

Next, STR and mtDNA assays were investigated. STRs were amplified and the number of alleles consistent with the handler was compared among the double swab method, cumulative swab method, and a consensus profile method that considered the five STR results from the individual casings in combination, to help distinguish real peaks from drop-in. For mtDNA, amplification parameters were optimized, segments between 220 and 270 base pairs in hypervariable regions I and II (HVI and HVII) were amplified, and the products underwent Sanger (dideoxy) sequencing. The mtDNA haplotypes produced were compared to those of the handlers in order to determine the utility of mtDNA analysis, and processing the spent cartridge casings individually and cumulatively.

Almost all of the samples, regardless of the swabbing method, produced few or no STR alleles, which was consistent with previous studies. In contrast, mtDNA was successfully amplified and sequenced from the vast majority of both individually and cumulatively swabbed spent cartridges, including those for which STR testing was negative. Given the increased sensitivity of mtDNA testing, contamination represents a particular problem, and extreme care is required to avoid it during evidence collection and processing. In spite of this caveat; however, mtDNA analysis from spent cartridge casings was quite successful, proving to be a more viable technique than STR typing of these samples.

Mitochondrial DNA, Cartridge Casings, DNA Collection

A162 Validation of a New Robotic System to Automate High Throughput mtDNA Sequencing in a Forensic Laboratory

Alexandra J. Mattioli, BS, Robert S. Oliver, MSc, and Kimberly S. Andreaggi, MFS, Dover AFB, 115 Purple Heart Dr, Dover, DE 19902*

After attending this presentation, attendees will understand the benefits and challenges encountered when validating a new robotic platform in a forensic laboratory for the mtDNA sequencing of high-quality reference samples.

This presentation will impact the forensic science community by providing information on how a robotic instrument can be successfully introduced into the laboratory and demonstrate how it can improve the high throughput processing workflow within the laboratory.

Currently, a number of forensic laboratories, including the Armed Forces DNA Identification Laboratory (AFDIL), are working toward developing novel automated solutions that allow cost effective, reproducible, and error-free processing of a high quantity of samples. Each year, the mitochondrial DNA (mtDNA) section receives and processes approximately 3,000 buccal swabs from maternal family members that are used for comparison to unknown samples from past U.S. military conflicts. Applying a high throughput approach toward reference processing is desired for increased efficiency and accuracy. For the last 12 years, AFDIL has used the Tecan Genesis® Workstation 200 to semi-automate all post-amplification mtDNA control region sequencing steps. This process, although accurate, required some user intervention due to a lack of fully integrated peripherals. In December of 2011, Tecan stopped supporting the older Genesis platforms. In preparation for this loss, AFDIL began the process to procure a new liquid handling instrument or face having to manually process family reference samples (FRS). It was vital that the new system continue to provide the necessary robotic capabilities while also incorporating enhancements that could further improve the workflow, increase overall sample throughput, and decrease user interactions.

Research and planning resulted in AFDIL acquiring and customizing the Hamilton's MICROLAB® STARplus liquid handling platform to allow for increased sample throughput and reduced user interactions during the automated mtDNA sequencing of FRS. The system includes eight independent pipetting channels, a 96-probe head, and two different gripper tools, as well as an external arm to integrate a plate sealer and eight thermal cyclers. Method development was an intensive process that included definition of labware, liquid class optimization, and the creation of a user-friendly workflow that allows parameters such as the number of samples or sequencing primers used to be easily changed. This flexibility ensures that the method can be utilized in a wide range of circumstances and by multiple sections at AFDIL without revisions. During method development, if any aspect of the automated post-amplification processing was unacceptable, the necessary modifications were made to the method or materials prior to the start of the validation. The development of a robust and reliable automated method was imperative to ensure efficient and accurate sample processing while meeting throughput requirements.

After five months of method development and optimization, an internal validation was performed to demonstrate that the Hamilton STARplus would produce sequencing products that were comparable to the results obtained when processed manually or on the Tecan. The validation included three experiments which were designed to meet applicable Quality Assurance Standards guidelines. Once the validation was complete, AFDIL's laboratory information management system and Standard Operating Procedures were updated for the integration of the new Hamilton robotic workflow. This post-amplification robotic system will enhance the high-throughput processing capabilities of reference samples and allow AFDIL to process at least 400 FRS per month, equating to over 6,000 sequencing reactions covering a 1,200-base pair region of the mtDNA genome. The implementation of the customized Hamilton STARPlus system into casework will decrease both processing time and required user interactions, greatly improving AFDIL's ability to process reference samples in real-time and ensure that the most up-to-date database is available for comparisons in missing persons cases.

The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of the Army, the Department of Defense, or the U.S. Government.

Mitochondrial DNA, Automation, Validation

A163 Detection of Low-Level DNA Sequences Associated With Nuclear Mitochondrial Pseudogenes (NumtS) From Human Mitochondrial Control Region Amplicons Using Massively Parallel 454 Pyrosequencing

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After attending this presentation, attendees will have a better understanding of next-generation sequencing (NGS) as applied to mitochondrial DNA control region analysis. Approaches to quantify minor variant detection thresholds of the Roche GS Junior 454 pyrosequencing instrument will be described. Additionally, the detection of nuclear pseudogenes during mixture study deep sequencing runs, and steps taken to confirm the presence of these pseudogenes in our donor population, will be discussed. Finally, detection of inconsistent, non-NumtS affiliated minor variants in hair shaft tissues will be discussed, and research approaches taken to elucidate causes of these variants.

This presentation will impact the forensic science community by outlining foundational research being performed in the area of Next-Generation Sequencing (NGS) of the human mtDNA control region. Preliminary suggestions for streamlined NGS sample preparation, use of Roche Amplicon Variant Analyzer (AVA) software, and data analysis considerations will be offered. Reasonable expectations for instrument detection threshold, pyrosequencing-specific sequencing errors, and tissue dependent differences in sequence data will be described. A per-run cost analysis using the Roche GS Junior platform will be presented.

Massively parallel pyrosequencing on the Roche GS Junior provides thousands of independent reads per sequencing run, and thus has the potential to detect and quantify minor variants with vastly greater sensitivity and precision than traditional Sanger sequencing. The minor variant detection threshold was determined for the Roche GS Junior instrument using mixtures of mtDNA hypervariable (HV) region amplicons with known sequences. In addition to the expected variants originally obtained using dideoxy terminator sequencing, a set of nineteen unexpected variants in HV1b reads (corresponding to base pairs 16159 – 16391 in the mitochondrial control region) at a level of approximately 1% were detected. These variants are reproducible and are always detected as a set within individual reads of HV1b amplicons. The total depth of coverage did not appear to affect the level at which the unexpected variants were detected. A standard nucleotide BLAST search of the variant sequence was performed which showed 100% sequence similarity to a segment of a 611bp nuclear mitochondrial pseudogene (NumtS) originally reported in 1995 by Zischler *et al*.[1] This NumtS is an insertion of the mitochondrial control region (bases 16,089 – 59) on the short arm of chromosome 11, spanning the primer binding sites of the targeted HV1b region.[2] Nuclear DNA specific primers flanking the insertion were used to amplify the pseudogene from buccal extracts without amplifying DNA from the mitochondrial control region.[3] This amplification strategy confirmed the presence of the NumtS in 19 out of 20 donors, with one donor being homozygous-negative for the insertion. Dideoxy terminator sequencing was used to successfully confirm the presence of the variant sequence in the amplified NumtS from donors positive for the insertion. This identification furthers the understanding of human mtDNA variants and is expected to have a positive effect on the interpretation of mtDNA profiles using deep sequencing methods in forensic casework. This work will ultimately allow expansion in the analysis of mtDNA beyond the control region using a whole genome amplification (WGA) technique to increase starting concentrations of extract from compromised samples.

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Mitochondrial DNA, Pyrosequencing, Variants

A164 Next Generation Sequencing Applications for SNPs and Mitochondrial DNA in Human Identification

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After attending this presentation, attendees will gain an understanding of comprehensive laboratory and bioinformatic workflows for obtaining mitochondrial and SNP genotypes using next-generation sequencing technology.

This presentation will impact the forensic science community by giving an understanding of the potential use of next generation sequencing for human identification applications.

Next-generation sequencing technology is a subject area in which the forensic community has previously done little research. However, this presentation shows that using genomic DNA or mitochondrial DNA extracted from bone, blood, buccal swab, or other forensic samples, one can obtain mitochondrial and/or SNP genotypes for up to 96 individuals in a single next-generation sequencing run.

The field of human identification has been dominated by capillary electrophoresis-based (CE) STR fragment analysis. There has also been a minor effort to sequence the hypervariable regions I/II of the mitochondrial genome by CE. The low throughput of CE sequencing makes it difficult to incorporate complex DNA testing into routine procedure for criminal labs. Next-generation DNA sequencing technologies have advanced dramatically in recent years, and the costs have been reduced enough to make adoption of next-generation sequencing more attractive to the forensic community and criminal labs. Laboratories that adopt a next-generation sequencing approach are capable of generating data that can be used to produce a more detailed genomic and phenotypic profile than the current STR approach at less cost than traditional sequencing or SNP assays.

The whole 16kb mitochondrial genome can be sequenced in one next-generation sequencing run. If sequenced on CE, 64 separate reactions would be necessary (assuming 500bp amplicons and forward/reverse sequences). In fact, the new higher throughput capacities enable whole mitochondrial genome sequencing of up to 96 individuals in a single sequencing run.

To test the feasibility of this idea, an assay system containing 32 fusion adaptors with a short sequence tag made of different combinations of nucleotides attached to an adaptor (barcode) was developed. The whole mitochondrial genome was amplified with 2 PCRs each yielding overlapping 8-9kb amplicons. The two PCR products were then combined, sheared, and ligated to an adapter and a fusion barcoded adaptor. The PCR products from each individual can then be pooled and sequenced in a single sequencing run. Additionally, for sequencing more compromised samples, a 2 PCR mitochondrial mini amplicon system consisting of two multiplexes of five primer sets spanning the mitochondrial control region was tested.

To demonstrate feasibilty for an SNP assay, a panel of 106 autosomal and 33 Y chromosome SNPs selected from publically available datasets was constructed. A single PCR multiplex for ~200bp amplicons covering the 139 SNP loci was generated. The PCR products were ligated to adaptors, and barcoded libraries from 32 individuals were pooled and sequenced in one sequencing run and compared to reference genotypes.

The high capacity of the next-generation sequencing technologies makes combining many of the currently used human identification methods possible. Multiplexes of any combination of STRs, phenotypic SNPs, autosomal identification SNPs, lineage SNPs, Y SNPs, Indels,

mitochondrial sequences, or SNPs are possible in one reaction. Inclusion of historical markers would allow for a gradual shift to other markers while at the same time keeping continuity with current forensic data sets.

Sequencing, Mitochondrial, SNP

A165 Estimating Genetic Ancestry Using SNP Analysis

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After attending this presentation, attendees will understand how utilizing SNP technology in a specific assay can be applied to crime scene evidence to obtain genetic ancestral information.

This presentation will impact the forensic science community by discovering new forensic DNA applications available for criminal investigations in helping to reduce the number of potential suspects in an unsolved or cold case.

The utilization of many worldwide DNA databases, such as CODIS, can be an essential tool in modern criminal investigations. Unfortunately, when an evidentiary DNA profile does not provide a viable suspect subsequent to a database search, the investigator may be left with little forensic direction. The use of ancestral testing can be a potential option to obtain additional information regarding the donor of DNA left at a crime scene. However, there are limitations to ancestry testing due to the potential that a person's overall ancestry may be wrongly assumed with the use of haplogroups. The mtDNA or YSTR testing methods are only evaluating a small, selected portion of a person's genomic ancestry. To assist in these critical situations, Sorenson Forensics developed Investigative LEAD™; a Single Nucleotide Polymorphism (SNP) based DNA test designed to estimate genetic ancestry against a model of five genetically distinct, putative parental populations. The populations and the reference samples representing them are as follows: Western European (HapMap CEU, Northwest European descent residing in Utah), West Sub-Saharan African (HapMap YRI, Yoruba from Ibadan, Nigeria), East Asian (HapMap CHB from Beijing, China), Indigenous American (compilation of samples identified as being from populations indigenous to North, Central, and South America including Maya, Pima, Karitiana, Surui, and Arawak descent), and the India Subcontinent (HapMap GIH, Gujarati Indian descent residing in Houston, TX). In addition, the Investigative LEAD assay covers all autosomal chromosomes and the test markers are associated with ancestral informativeness so that an accurate representation of the human genetic anthropology is obtained. Our method uses 190 SNP Ancestry Informative Markers (AIMs) chosen from their scored ability to specifically differentiate between the five reference populations using Principal Component Analysis (PCA) as the comparative analysis tool and includes some markers identified as informative in previous genetic ancestry estimation publications. The method uses fluorescence-based polymerase chain reaction reagents to provide qualitative detection of targets using post-PCR endpoint analysis. As a modified approach to standard genotyping, this system miniaturizes the reactions down to 33 nanoliters for cost efficiency and high throughput. The data analysis uses a Principal Component Analysis (PCA) and a proprietary algorithm based on the program FRAPPE. The method calculates affinity levels of an individual DNA sample and then compares that to at least a hundred randomly selected subsets of individuals from the reference populations. Background interference is calculated simultaneously and is used to estimate confidence intervals based on a calibration that was effected using thousands of worldwide individuals. The effects of inhibitors, species specificity, sensitivity, non-probative casework samples, and a comparison of extraction methods from the developmental validation will be presented to demonstrate that the test is robust and viable for the forensic sample types frequently encountered in criminal investigations. The test is capable of providing valuable information regarding the genetic ancestry of the donor of crime scene DNA evidence, which can subsequently aid in reducing a pool of suspects in the investigation. Another application for this technology will be to assist in the determination of the possible contributor of skeletal remains.

SNP, Analysis, DNA

A166 Implementing SNPs Into Forensic Casework: An Assay and Interpretation Models to Predict Ancestry and Eye Color

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After attending this presentation, attendees will be informed of a new Single Nucleotide Polymorphism (SNP) assay that can be used to predict ancestry and eye color of an unknown individual, also covering developmental validation, steps of individual lab implementation, and interpretation of results.

This presentation will impact the forensic science community by understanding that when an STR DNA profile obtained from crime scene evidence is not consistent with known or database profiles, no further information is available from genetic evidence. If forensic laboratories were to implement this assay, then further analysis targeted at inferring the ancestral origin and phenotypic characteristics of the perpetrator could provide valuable investigative information. Results of this analysis would aid in prioritizing suspect processing, corroborating witness testimony, determining the relevance of evidence to a crime, and ultimately increase the ability to identify individuals related to the crime scene.

The existence of population-specific SNP variation found throughout the world can be used to predict the ancestry of an unknown individual, and, in some cases, can also predict externally visible characteristics (EVC). A panel of 50 SNPs showing strong association to pigmentation phenotypes and/or ancestry has recently been determined and an assay with which to type these SNPs using the single base extension (SBE) technology has been developed. Evaluation of this panel found it to be sensitive and robust, similar to current STR typing methods. All the primer sequences, protocols, and analysis parameters (including GeneMapper ID and GeneMarker panels/bins) are publicly available. This panel can be implemented with minimal investment, as it uses the same equipment and similar methods already in use in forensic DNA casework laboratories.

Also to aid in implementation, an ancestry interpretation method has been made available, using a U.S. sample set and the open source web-based application Snipper. By adding the genotypes from an unknown sample and running Snipper with the known sample set, users will receive likelihood ratio results, which have been shown to reliably predict the ancestry of an unknown individual. Further, if the individual is determined to be of European ancestry, results of this evaluation show that a freely available excel-based prediction model can be used to indicate eye color. The 50 SNPs selected were also based on their ability to provide information on hair color and skin melanin content; however, at this time the number of individuals with known phenotype and corresponding genotype data for these SNPs is insufficient to generate reliable prediction models. Funding to increase the size of this U.S. sample database is being sought.

Lastly, evaluations are ongoing to determine if other forensic DNA results (STR, YSTR, mtDNA) can be integrated to increase the ancestry prediction ability. Using the free, excel-based Omnipop, a method of evaluating the random match probabilities of an individual's STR profile in different populations that could be used to predict ancestry has been developed. Also under development is the incorporation of the YSTR haplotype worldwide distribution using a web-based haplogroup predictor. These methods have been tested on a U.S. sample set to determine if combining results from other markers strengthens or weakens the SNP ancestry prediction. Overall, such a model could be incorporated into forensic casework to maximize the information obtained from an unknown sample.

SNP, Ancestry, Phenotype

A167 Validation of IrisPlex in the United States: A DNA-Based Tool for the Prediction of Eye Color

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After attending this presentation, attendees will understand the concept of DNA phenotyping, especially relating to iris color, the application of the IrisPlex system and its prediction model component, alternative approaches for quantifying iris color, and other possible prediction methods.

This presentation will impact the forensic science community by providing information regarding casework where DNA from biological evidence is limited in providing probative information for the investigation. Physical characteristics of the donor such as hair, skin, or eye color, may present investigational leads when the conventional STR profile of the unknown DNA sample does not match any suspects or victims nor hits in a DNA database. It may also be useful for the identification of missing persons or victims of mass disasters to provide investigators with the most likely appearance of the unknown individual. S DNA phenotyping is the ability to determine physical external characteristics solely based on genotype analysis. Iris color is a complex genetic trait determined by several different genes resulting in highly polymorphic phenotypes. The IrisPlex system was developed in the Netherlands and is a multiplex SNP genotyping assay combined with a statistical model for predicting eye color. The system targets six eye color-informative, Single Nucleotide Polymorphisms (SNP) and using the genotype data, a multinomial logistic regression model was developed based on minor allele frequencies and is used for predicting the probability of eye color into three categories: brown, blue, and intermediate.

This work focuses on the developmental validation of the IrisPlex system and to evaluate the accuracy of the system's prediction component on a North American population. Validation of this work was done amplifying the same SNPs using the SNaPshot chemistry single base extension method. Capillary electrophoresis was performed on the ABI 3500 genetic analyzer.

The results of the IrisPlex study indicated predictions with greater than 90% accuracy of blue and brown eye color based on a European (Dutch) population. The prediction results based on the North American sample population so far, using the same parameters in the IrisPlex model, do not have the same level of accuracy or prediction power, especially with the blue color. All but one sample tested from the North American population so far is heterozygous at the rs12913832 SNP locus, which has been found to have the highest association with blue and brown eye color predictions when in the homozygous state. The highest probability predicted with the North American samples collected so far is 56% for blue eye color. These results are reasonably expected as North America is a highly admixed population compared to the European populations previously studied, which is where most eye color variation originates.

The parameters of the statistical regression model need to be adjusted for the differences in the allelic and genotypic frequencies between the two populations, and this is one of the goals of this work as a way of improving the prediction accuracy. In addition to validating the method using a North American population, other methods for prediction and quantitation of iris color, possibly to achieve more accuracy with the intermediate color category, are considered and evaluated. One such method for eye color classification is quantification based on separation of the iris color into red, green, and blue (RGB) and luminosity values to create a numerical color scale. Association of that value with the genotype data may be used for prediction purposes.

Iris Color, SNPs, IrisPlex

A168 Alternative Methods for Human Identification: DNA Base Composition Profiling by Electrospray Ionization Time-of-Flight Mass Spectrometry

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After attending this presentation, attendees will be familiar with the methods used for forensic identification of mitochondrial DNA (mtDNA) by electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) and the capabilities of the Plex-ID instrument designed for this purpose. The research findings can guide forensic experts in practical aspects of adopting mass spectrometry as a technique for use in human identification by analysis of the mtDNA control region.

This presentation will impact the forensics science community by delineating the performance capabilities of mass spectrometry-based mitochondrial DNA base composition profiling. A full understanding of the capabilities of ESI-TOF MS instrumentation is critical to the adoption of the technology in the forensic sciences so that cost and labor advantages can be gained without exceeding expectations of performance in routine casework.

Mass spectrometry base composition profiling represents an alternative technology for forensic identification of biological material via mtDNA with improvements over the current method of DNA sequencing through reductions in cost, labor, and time required to generate a result. Mass spectrometry-based detection of DNA is not hindered by length heteroplasmy or mixed contributor samples, both of which are challenging to analyze by DNA sequencing.

In order to investigate the suitability of the Plex-ID ESI-TOF MS for use in forensic identification, experiments have been performed at NIST to assess the operational characteristics of the system when used for base composition profiling of mtDNA. Research aimed to assess the function of the Plex-ID ESI-TOF MS in the areas of: (1) concordance with Sanger sequencing data; (2) limits of detection; (3) monitoring for contamination; and, (4) ability to detect mixtures. Concordance studies evaluated results from the Plex-ID against Sanger sequencing data for DNA templates originating from four major population segments in the United States: Caucasian, African American, Hispanic American, and Asian American. PCR sensitivity was assessed through serial dilution of three unique DNA templates. Sensitivity was monitored over time to ascertain whether there may be changes in sensitivity levels of the PCR reagents or the instrument. Contamination was assessed through an experiment designed to evaluate multiple potential sources of contaminants. The ability to detect and successfully identify mixtures of DNA templates was gauged by mixing two templates together at ratios of 99:1, 19:1, 9:1, 3:1, 1:1, 1:3, 1:9, 1:19, and 1:99 and co-amplifying the templates for subsequent detection on the mass spectrometer.

Concordance study results from 669 samples identified four samples which did not generate full profiles of 24 amplicons, with a single amplicon failing to be detected. This yields a concordance rate of 99.4% (665/669) when using the criteria that a full profile is necessary for registration with the instrument database. When comparing the number of successfully measured amplicon masses, the concordance rate is 99.9% (16,052/16,056). Importantly, the discordances were limited to incomplete profiles rather than incorrect measurements. The limit of detection of template DNA was found to be well below the manufacturer's suggested minimum quantity of 200pg per sample, divided into eight multiplex PCR reactions. Full mtDNA profiles were generated over the range of 8 to 40pg of nuclear DNA template. Sensitivity levels remained within acceptable limits with slight fluctuations during four months of monitoring. Samples were variable in their mtDNA copy number, as expected. To monitor for contamination, 18 experiments were run over the course of eight months. No contamination was detected. Mixtures could be consistently detected when the two components were present at 3:1, 1:1, or 1:3 ratios.

However, complete profiles for both mixture components could not reliably be generated due to limitations in the instrument's ability to resolve two masses that are within 11 Daltons of each other.

Savings in cost and labor inputs, combined with high levels of sensitivity and accuracy make, the ESI-TOF mass spectrometry technique well suited to the forensic human identification laboratory.

A full understanding of the capabilities of ESI-TOF MS instrumentation is critical to the adoption of the technology in the forensic sciences so that cost and labor advantages can be gained without exceeding expectations of performance in routine casework. This presentation will impact the forensic science community by delineating the performance capabilities of mass spectrometry-based mitochondrial DNA base composition profiling.

Mass Spectrometry, Base Composition, Mitochondrial DNA

A169 Evaluation of Pressure Cycling Treatment on Barocycler® NEP3229 for Extraction of Low Template Forensic DNA Samples

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After attending this presentation, attendees will understand the efforts to increase recovery of DNA from low template samples through the use of Pressure Cycle Treatment (PCT) with the Barocycler® NEP3229 (Pressure BioSciences). Specific goals were addressed, including simultaneous incubation of samples, maximization of DNA yield, and evaluation of instrument parameters and consumables for the target sample. An existing QIAGEN protocol was compared to a modified version incorporating pressure cycling pretreatment.

This presentation will impact the forensic science community by providing laboratories with additional knowledge to make informed decisions when evaluating pressure cycling strategies for challenging samples, such as those collected from touched items, hair, bone, and teeth.

Technological advancements have greatly increased the sensitivity of DNA testing in recent years. Techniques for low template autosomal DNA testing focus, primarily, on extending amplification cycles or increasing detection of the amplified products on capillary electrophoresis via reduction of salts and unincorporated primers. The class of low template samples initially targeted for this study, those recovered from touched items, are generally collected on a cotton swab substrate. Labs have long been challenged with more efficient removal of DNA sample from this substrate. Swabbing solutions, substrate material, and incubation methods have been examined in efforts to improve recovery.

Pressure cycling has recently emerged as a technique with the potential to improve DNA yield prior to amplification, with or without adjustments to amplification or clean-up of amplification products. High pressure treatment of samples prior to extraction has been hypothesized to compromise plasma membranes leading to an increase in permeability of extraction reagents and to more efficient cell lysis. In addition, cyclical exposure of samples to alternating high and ambient pressures may improve the removal of cells and cell-free DNA from the substrate. Within the FT-500ND PULSE tubes, a ram generates a pressure differential inside a tube, forcing the buffer through the swab substrate. This action may improve DNA release from the substrate and/or cell lysis in some fashion.

The present study was undertaken to evaluate a pre-amplification technique with the potential to improve recovery of DNA from a commonly used swab substrate and subsequent extraction. Various parameters were evaluated including incubation method, cycle number, and time at maximum pressure. Samples were prepared by adding 1,000pg, 500pg, 250pg, and 100pg of DNA from a calibrated solution of diluted human saliva to one-half of a cotton swab and drying overnight. Incubation was performed in PULSE tubes at 56°C within the Barocycler® NEP3229 chamber and under a series of experimental conditions including 20, 40, 60, or 80 cycles alternating between 20, 40, 60, or 80 seconds at 35k psi and 10 seconds at ambient pressure. Samples were quantified using Quantifiler® Duo (Applied BioSystems) and amplified using AmpFℓSTR® Identifiler® Plus (Applied BioSystems) reagents at 28 cycles. The amplicons were then injected onto a 3130*xl* genetic analyzer (Applied BioSystems).

Results from the various Barocycler® NEP3229 run parameters tested were varied depending on the specific target amount of DNA in the sample. Optimal run parameters were selected based on an improvement in performance across all low template DNA targets. For the DNA amounts tested, 1,000pg, 500pg, 250pg, and 100pg, runs of 20 cycles with 20 second intervals at maximum pressure recovered more DNA than the existing method across all four DNA target amounts with 22.8% (1,000pg), 50.5% (500pg), 59.3% (250pg), and 56.2% (100pg) improvements. At 1,000pg and 500pg amounts of target DNA, the 80-cycle 20 second method performed slightly better, with additional 23.0% (1,000pg) and 16.8% (500pg) increases. However, this method did not have the same affect at the 250 pg and 100 pg target amounts of DNA, recovering 45.8% (250pg) and 79.8% (100pg) less DNA than the 20-cycle 20 second method. These data agree with recoveries from 1uL of human saliva. Samples with higher amounts of DNA do benefit from increasing the number of PCT cycles; however, samples containing lower amounts of DNA appear to require fewer cycles to remove the sample from the substrate, perhaps preventing re-adherence to fibers of the cotton swab.

Findings from additional validation studies performed using pressure cycling pretreatment as well as its affect on DNA recovery from bone, hair, and tooth samples will be shared.

Pressure Cycling, Low Template, Extraction

A170 A 27-Locus STR Assay to Meet All United States and European Law Enforcement Agency Standards

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After attending this presentation, attendees will become familiar with a 27-locus, six-dye multiplex containing all STR loci recommended or proposed for use in national databases in the United States, Europe, and Asia, and with advanced instrumentation that permits separation and detection of up to eight separate dyes to support even more highly multiplexed assays.

This presentation will impact the forensic science community by introducing an STR multiplex containing all existing and proposed standard loci for database searches in United States, Europe, and Asia that will support cross-border searching of all existing and proposed databases.

Investigative and judicial agencies worldwide have developed DNA databases to store short tandem repeat (STR) profiles. These databases provide capabilities to match DNA profiles from crime scene samples to database entries, provide investigative leads, and permit familial searching. Recently the FBI proposed expansion of the CODIS core loci to include amelogenin plus 19 required STR loci and three recommended STR loci. This approach retains the current 13 CODIS core STR loci (with transfer of the locus TPOX from required to recommended status), while expanding the set to include STR loci popular in other parts of the world, notably Europe. Increasing the number of loci in multiplex sets will not only permit compatibility across borders, but will also diminish the chances of obtaining false hits in databases, and will enhance the ability to perform familial searching. It will also improve kinship analysis in support of immigration requests.

In this presentation, attendees will become familiar with the development of a multiplex STR set permitting co-amplification of 26 STR loci plus amelogenin to supporting expansion of database compatibility across national and international borders. The 27plex set includes all 15 loci in common between the proposed CODIS required

core and the European standard, i.e., amelogenin, D1S1656, D2S441, D2S1338, D3S1358, D8S1179, D10S1248, D12S391, D16S539, D18S51, D19S433, D21S11, FGA, TH01, and vWA. In addition, the 27plex includes locus D22S1045 that is part of the European standard and is recommended in the proposed expanded CODIS core. It includes the proposed five additional required CODIS core loci CSF1PO, D5S818, D7S820, D13S317, and DYS391, and the remaining two proposed CODIS recommended loci SE33, and TPOX. Beyond these, the multiplex contains an additional locus commonly employed in Asia, D6S1043, and three other loci, Penta C, Penta D, and Penta E, respectively, that are commercially available and are used in many database searches.

To maintain the forensic preference for amplification products of less than 500 bases while incorporating so many highly polymorphic loci required expansion of previously described dye panels to six fluorescent dyes and modification of the existing NetBio GeneBench electrophoresis instrument to permit detection, separation, and display of amplified products containing each individual dye. In addition, the multiplex design generates amplified products of all alleles smaller than 400 bases except for the least commonly employed loci D6S1043, SE33, Penta C, Penta D, and Penta E. The authors propose the use of the 27-locus multiplex to permit laboratories in any jurisdiction to employ an expanded common global STR profiling set to permit full application of all data currently available in any of the national or international databases.

The multiplex has been optimized to generate all products in a 19.5 minute amplification reaction. The modified instrument used to evaluate the amplified products can simultaneously detect and employ software to separate eight or more different fluorescent dyes. These additional advances provide opportunity for adaptations to Rapid DNA Analysis and to multiple instrument platforms, and for the creation of a variety of amplification formats from higher order multiplexes to highly discriminating miniplexes.

Multiplex, CODIS, Database

A171 Detection of Deletion/Insertion Polymorphisms From Challenged Samples

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After attending this presentation, attendees will learn an alternate method of detecting polymorphism from environmentally insulted evidence samples.

This presentation will impact the forensic science community by allowing the scientist to generate insertion/deletion polymorphism profiles from samples where complete Short Tandem Repeat (STR) DNA profiles may not be possible to obtain.

Analysis of Short Tandem Repeats (STRs) is currently the most commonly used method for human identification. However, DNA extracted from evidence samples exposed to environmental insults does not always yield complete STR profiles. Light, humidity, elevated temperatures, and bacterial or fungal contaminants all degrade DNA, which in turn can lead to the loss of genetic information. Also, the efficiency of the PCR amplification process is reduced when inhibitors such as salts, heme in blood, indigo dye found in denim, phenolic compounds, melanin found in skin and hair, humic acid from soil, and collagen and calcium in bone are present in the extracted DNA. Degradation and inhibition can lead to loss of signal, peak imbalance, and allelic dropout with current STR technology. In situations where DNA is highly degraded, the molecule becomes fragmented and the chances of obtaining complete profiles are reduced. Typically, the larger amplicons are the first to fall below the detection limit. This problem has prompted research in the area of extraction and amplification methods to obtain complete DNA profiles from these types of compromised samples.

The Investigator DIPplex® Kit from Qiagen combines 30 insertions and deletions (InDels) markers as well as Amelogenin in a single PCR reaction. These unlinked markers are distributed across 19 chromosomes. One significant advantage of this kit is that stutter peaks commonly seen as artifacts of STR analysis do not appear in DIPplex® profiles. The PCR products generated by the primers are no larger than 150 base pairs. Due to the small amplicon size, the assay is highly sensitive and the manufacturer claims it can create a full DNA profile from as little as 63pg of DNA.

The current study focuses on the detection of insertion/deletion polymorphisms from challenged samples using the Investigator DIPplex® kit from Qiagen. Unlike the PCR amplification kits currently available in the forensic community that amplify 15 or more STR loci, the DIPplex® kit allows for multiplex amplification of 30 bi-allelic areas of known InDels plus the Amelogenin locus. This PCR amplification kit uses reduced amplicon sizes (maximum of 150bp), similar to SNPs, improving the amplification of degraded samples. The combination of current STR analysis procedures and small amplicon sizes makes InDels suitable for pristine as well as degraded DNA evidence samples.

This research included analyzing different types of body fluids from humans, some of which have also been subjected to environmental insults. Body fluids such as saliva, blood, semen, and nasal secretions, etc. from male and female human donors were analyzed to determine if they yield consistent profiles from the same donor. Another goal of this research was to assess the DIPplex® kit's capacity for samples that closely resemble forensic casework evidence. For the purpose of validating the kit, samples from various animals were also included to determine if the kit is species specific.

The experiments indicated that complete DIPplex® profiles can be obtained from degraded samples which yielded either partial or no STR profiles. These samples included washed bloodstains deposited on various types of fabrics, such as denim.

Reducing the primer concentration as well as the total reaction volume gave results comparable to profiles obtained when following the manufacturer's recommended protocol. Increasing the cycle number from the recommended protocol also improved the yield of profiles from DNA extracts which were degraded and below optimum quantity.

Direct amplification with 1.2mm blood and saliva punches from various substrates yielded complete profiles when using less than the recommended cycle number.

DNA extracted from several animals yielded either no profile or profiles dissimilar to humans. The results indicate that analysis of bi-allelic insertion/deletion polymorphisms can be useful in supplementing data obtained with STR profiles.

InDels, DNA Polymorphism, STR

A172 Determination and Use of Optimized Techniques to Analyze Trace DNA From Fingernails

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After attending this presentation, attendees will recognize optimal methods for collection and processing of fingernail evidence and learn the importance these methods have for forensic practitioners.

This presentation will impact the forensic science community by introducing the best ways to collect and process trace evidence from fingernails in order to obtain the highest yields of exogenous DNA, along with the most genetic data. The results of this research have the potential to change the way forensic nurses, pathologists, and forensic biologists collect and process trace evidence from fingernails.

When two or more individuals come into contact with one another during a physical assault, a victim may fight back against the assailant, potentially resulting in the transfer of biological material, which has the potential to act as important evidence. In particular, a person struggling to free themselves from an assailant may grab and scratch that person,

resulting in the deposition of cells beneath their fingernails. Given this, medical examiners, sexual assault nurse examiners, and others regularly collect fingernail evidence following assault, however, the manner in which they do so is highly variable, and the success in obtaining probative evidence is unknown.

Currently, various procedures are used for collecting biological evide nce from nails, including clipping the nail itself, scraping beneath the nail using a wooden applicator, and swabbing underneath the nail. In the first portion of this research, female volunteers donated fingernails, on which one microliter of blood from a male volunteer was deposited. DNA was isolated using an organic extraction, and male specific DNA was quantified using real-time PCR. Soaking the nails directly in digestion buffer yielded the highest levels of male DNA, while scraping recovered the least.

Next, different extraction methods were compared in order to optimize yields; a silica-based kit extraction was compared to the organic extraction, with the former yielding more DNA. Several aspects of the kit extraction were then optimized, including incorporating more than one elution and the volume used for elution.

Genetic analyses included comparing autosomal STRs and Y chromosome STRs. The soaking and swabbing procedures resulted in mixtures of nail and blood profiles, while the scraping procedure generally showed only male DNA, although allelic dropout often occurred. Assaying Y chromosome STRs produced clean, complete profiles from both soaked and swabbed nails, while scrapings again displayed dropout.

Crime laboratories often receive nails as a set, and process them together using a cumulative swab, in order to save time and resources. This has the potential to transfer material from a nail that harbors exogenous DNA to ones that do not, resulting in cross contamination and/or loss of valuable evidence. Male blood was placed on one or two female nails, which were then swabbed intermittent with nails without blood deposition. This cumulative swabbing led to cross contamination between nails, including full Y chromosome STR profiles from the nails without blood. In contrast, when nails with and without blood were transported together and then processed individually, no cross contamination occurred, although a substantial amount of male DNA was lost through transportation.

Finally, the fully optimized procedure was tested on actual scratchings. Female volunteers scratched male volunteers on the underside of the forearm, using the middle three fingers. The scale was tared with the forearm relaxed on the center, and the set amount of force was applied before scratching commenced. The nails were clipped or swabbed, and the resultant nail or swab head was soaked in the kit's digestion buffer overnight. Multiple elutions were used to recover DNA from the kit columns. Male DNA was quantified, and DNAs were assayed for both autosomal STRs and Y chromosome STRs.

Fingernails, DNA Analysis, Y-STRs

A173 Development of a Highly Sensitive Quantification System for Assessing DNA Quality in Forensic Samples

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After attending this presentation, attendees will understand the scientific basis for primer and probe design of a human DNA test method that can simultaneously estimate the quantity and quality of DNA in a potentially degraded forensic sample.

The presentation will impact the forensic science community by providing forensic DNA analysts with information regarding a new DNA quality and quantity assessment method, a highly sensitive system and valuable tool when making decisions regarding the appropriate test kit to analyze potentially degraded forensic DNA samples.

Real-time PCR provides reliable results which are essential for determining the amount of amplifiable DNA in a biological sample. The recent advances in mini STR analysis systems have now made it possible to analyze highly compromised samples. A system which can assess the extent of degradation in a forensic sample will be a useful tool for DNA analysts. Recent scientific literature reports the evaluation of the quality assessment of degraded DNA samples using Ya5-lineage Alu genetic element.[1] This presentation utilizes two independent genomic targets to obtain quantification of an 80bp short DNA fragment and a 250bp long DNA fragment in a degraded DNA sample. A multi-copy intra *Alu* based approach, to quantify human specific DNA in an evidence sample, has been successfully used to obtain DNA quantification with high sensitivity.[2] *Alu* are Short Interspersed Elements (SINE), approximately 300bp insertions which are distributed throughout the human genome in large copy number. The use of an internal primer to amplify a segment of an *Alu* element allows for human specificity as well as high sensitivity when compared to a single copy target. In this study, primers and probes were designed using two independent intra *Alu* insertions targets. The 80bp target sequence is from aYb8-lineage specific *Alu* insertion, whereas the 250bp target sequence is from Ya5-lineage *Alu* insertion. Use of a multi-copy target, two different size Alu markers along with a synthetic target, as an Internal Positive Control (IPC), provides an additional assessment for the presence of PCR inhibitors in the test sample.

The average age of Yb – lineage subfamily is estimated as 2.39 million years. It is estimated that the human genome contains over 1800 *Alu* Yb family elements and, out of those, approximately 50% are from the Yb8 subfamily. Another advantage of the *Alu* Yb 8 system is the presence of a large number of fixed insertions. It has been reported that only 20% of the Yb-lineage *Alu* elements are polymorphic for insertion presence or absence in the human genome.[3] Because a large number of these fixed elements are present in every human genome, this minimizes the individual specific variation possible when using a multi-copy target quantification system.

The precision and sensitivity studies indicated that this system has a sensitivity threshold in the range of 3-4pg, similar to those reported for other *Alu* based quantification systems. The slope of the standard curve ranged between -3.53 and -3.3. The amount of synthetic IPC target was adjusted to provide reproducible Ct values between 12-14 cycles for samples with no inhibition. A correlation study of estimated quantification for both 80bp and 250bp fragments with the STR analysis results obtained from DNAse I degraded DNA samples will be presented.

In conclusion, a DNA-based qualitative/quantitative/inhibition assessment system, that accurately predicts the status of a biological sample, will be a valuable tool for deciding which DNA test kit to utilize when processing forensically compromised samples for DNA testing.

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DNA Degradation, DNA Quantification, DNA Quality

A174 Recertification of Standard Reference Material 2372 – the Human DNA Quantitation Standard: The What, the Why, and the How

Margaret C. Kline, MS, Erica Butts, MFS, and David L. Duewer, PhD, NIST, 100 Bureau Dr, MS 8314, Gaithersburg, MD 20899-8314*

After attending this presentation, attendees will understand why the National Institute of Standards and Technology (NIST) has recertified the standard reference material 2372 (SRM 2372) human DNA quantitation standard, and how that recertification has been performed to achieve traceability to SI units.

This presentation will impact the forensic science community by assuring the understanding of procedures and policies followed by NIST to assure the quality and fitness-for-purpose of the standard reference materials that the community uses to assure the quality of their measurements and to demonstrate that quality to their accrediting bodies.

SRM 2372 is intended primarily for use in the value assignment of human genomic deoxyribonucleic acid (DNA) forensic quantitation 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. SRM 2372 was originally certified for spectroscopic traceability in units of decadic attenuance, D_{10}. The D_{10} scale is a measure of absorbance and is traceable to the unit 1. The certified values of all three SRM 2372 components had D_{10} values of approximately one. The conventional conversion factor for aqueous solutions of double stranded DNA (dsDNA) is: 1.0 D_{10} at 260nm = 50ng/µL DNA. Data on the performance of the SRM 2372 components with three qPCR methods is supplied at http://www.cstl.nist.gov/biotech/strbase/srm2372.htm.

The genomic DNA in all three of the SRM 2372 components was prepared to be dsDNA; however, five years after production these dsDNA solutions transformed into mixtures of dsDNA and single stranded DNA (ssDNA). The conventional conversion factor for aqueous solutions of ssDNA is: 1.0 D_{10} at 260 nm = 40ng/µL DNA. Therefore, this partial conformational change increased D_{10} at 260nm but did not change the DNA mass concentrations or the behavior of the materials in qPCR assays. However, recall that the SRM 2372 components were certified for their spectroscopic properties and not for the conventional conversion to mass concentration. Sales of SRM 2372 were therefore suspended as soon as the spectroscopic instability was recognized.

After determining that the solution volumes of the SRM 2372 components were unchanged and there had been no appreciable degradation of the DNA, it was decided to recertify the remaining units for the spectroscopic properties of ssDNA. Since the conversion between dsDNA and ssDNA was incomplete and somewhat variable among units, methods for users of these materials to force complete conversion to ssDNA are required. Two methods have been developed: heat treatment at 98°C and strand disassociation by sodium hydroxide (NaOH). The recertified values incorporate the variability of this conversion and, as such, the uncertainty intervals for the new values are larger than the original ones. The re-certified spectroscopic values remain traceable to the unit 1.

In addition, the SRM 2372 components are now certified for "copy number," that is, the total number of genome-equivalents present in the solution based on results from numerous digital PCR assays. qPCR assays associated with chromosomes 1, 4, 5, 6, 9, and 14 were developed in-house to be able to assess possible copy number variability issues associated with the three SRM components. Once the qPCR assays were optimized using an AB7500, they were then transposed onto the digital PCR platforms for analysis of copy number. Historically, component C of SRM 2372 has shown variability in some commercial qPCR assays. Developing qPCR assays that cover areas of six different chromosomes was done to try and understand the variability associated with these past results.

SRM 2372 components have also been checked for their performance with commercially available qPCR assays that may be of interest to the forensic DNA community.

DNA Quantitation, Digital PCR, Decadic Attenuance

A175 Expansion of the ILRC Database: Addition of Weathered and Biologically Degraded Liquids

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The goal of this presentation is to inform attendees of the addition of degraded ignitable liquids to the Ignitable Liquids Reference Collection (ILRC) database.

This presentation will impact the forensic science community by providing information on the degradation of ignitable liquids through evaporation (weathering) and biological degradation. The ILRC database will be modified to incorporate degradation data of fifty ignitable liquids.

In 2000, the Ignitable Liquids Reference Collection (ILRC) and Database were established as a joint project between the National Center for Forensic Science (NCFS) and the Technical Working Group for Fire and Explosions (TWGFEX). The need for a collection of reference ignitable liquids with associated GC/MS analysis data was confirmed by fire debris analysts in a 1998 national survey of forensic laboratories.[1] In 2002, the ILRC database became accessible to the public. The collection of reference ignitable liquids and the database of GC-MS analysis data are housed at NCFS. The ILRC committee, a committee within TWGFEX, reviews all of the data and classifies each reference ignitable liquid based on the American Society for Testing and Materials (ASTM) E1618 classification scheme.[2] The ILRC database contains product information, classification information, and GC/MS data.

In 2007, the ILRC database software was upgraded to provide the user enhanced searching capabilities. In 2010, NCFS and TWGFEX developed a substrate database containing a compilation of headspace GC/MS data from burned and unburned materials that are common to fire scenes. Substrates are materials which undergo pyrolysis and combustion processes during a fire and are constituents in fire debris collected at a fire scene. These materials may produce compounds that can interfere with the identification of ignitable liquids in fire debris. The database can assist fire debris analysts by demonstrating the types of compounds and chromatographic patterns that may be produced by these commonly encountered materials.

Processes that complicate the analysis of ignitable liquid residues in fire debris, besides the interfering products from the pyrolysis and combustion of substrate materials, is evaporation and biological degradation. Weathering is the evaporation of the more volatile compounds of an ignitable liquid resulting in a greater concentration of the less volatile compounds.[3] Ignitable liquid residues recovered from soils or other organic matter are known to lack target compounds that are associated with common types of ignitable liquids due to the consumption of the ignitable liquid residue by microorganisms.[4] While evaporation affects the chromatographic profile, primarily through loss of the "front end" of the chromatographic profile by evaporation of the most volatile components, biological degradation tends to selectively remove certain types of hydrocarbons.

The National Center for Forensic Science (NCFS), Indiana University – Purdue University Indianapolis (IUPUI) and the ILRC committee of the Technical Working Group for Fire and Explosions are collaborating to integrate weathered and biologically degraded ignitable liquids into the database. Ignitable liquids will be weathered to 25%, 50%, 75%, 90%, and 95% and biologically degraded at zero, seven, fourteen, and twenty-one day time periods. The database software will be modified to enable the user to search for the additional ignitable liquids.

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the Government, and no official endorsement should be inferred. Support is also acknowledged from the University of Central Florida, National Center for Forensic Science, a State of Florida Type II Research Center.

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Fire Debris, Ignitable Liquids, Database

A176 Comparison of SIMCA With LDA and QDA for the Identification and Classification of Ignitable Liquid Residues

Erin E. Waddell, BS, Mary R. Williams, BS, and Michael E. Sigman, PhD, Univ of Central Florida, NCFS, PO Box 162367, Orlando, FL 32816*

The goal of this presentation is to establish a methodology with known error rates for identification and classification of ignitable liquids in fire debris samples.

This presentation will impact the forensic science community by discussing the benefits of each method when applied to ignitable liquid and pyrolysis product classification in fire debris analysis. The methods investigated are intended to provide statistical support for current laboratory practices.

This research compared chemometric methods for classifying ignitable liquids and ignitable liquid residues in fire debris samples. The methods emphasized identifying samples that contain ignitable liquid residues and assigning ignitable liquid residues to classes as defined by the American Society for Testing and Materials (ASTM) E1618 standard. ASTM classes include: aromatic, gasoline, petroleum distillates, isoparaffinics, naphthenic paraffinics, normal alkanes, miscellaneous, and oxygenates. This presentation will impact the forensic science community by discussing the benefits of each method when applied to ignitable liquid and pyrolysis product classification in fire debris analysis. The methods investigated are intended to provide statistical support for current laboratory practices. The goal of this research is to establish a methodology with known error rates for the identification and classification of ignitable liquids in fire debris samples.

Results using Soft Independent Modeling Of Class Analogies (SIMCA) will be compared to those obtained using Linear Discriminant Analysis (LDA) and Quadratic Discriminant Analysis (QDA). SIMCA is a soft classification technique which allows a sample to be classified into a single class, multiple classes, or to be unassigned. The option of classifying into multiple classes could be beneficial for fire debris samples since the ratio of ignitable liquid residue-to-substrate pyrolysis is unknown. Discriminant analysis is a hard classification technique which means that each sample must be assigned to a single class and failure to assign to a class is not an option. For both techniques, models were developed from libraries of Gas Chromatography-Mass Spectrometry (GC/MS) data for ignitable liquids and pyrolysis products. Models for assigning samples to the ASTM classes were based on the Total Ion Spectrum (TIS). Cross-validation techniques were also used for each chemometric method. A test set was created by randomly selecting 20% of the samples from each class, with the remaining 80% developing a model data set. The samples in the test set were then classified using the respective chemometric method. Cross-validation steps were repeated 100 times with a new test set being selected and classified each time. Total correct classification percentages were calculated as the sum of the cross-validation tests. All models developed for ignitable liquid and substrate library data were applied to fire debris samples.

Outlier samples within each class were first removed based on their orthogonal and score distances before the SIMCA model was developed. For each class, Principal Components Analysis (PCA) was performed and the cutoff distances for the orthogonal and score distances were calculated from the model data. The test data set was projected into the model PCA space for the respective classes and orthogonal and score distances were calculated. A sample was assigned to the given class if the calculated score value was less than or equal to one. If the value exceeded one, the sample was not assigned to that class. A sample was considered to be correct if it only assigned to the known class and partially correct if it assigned to multiple classes that included the correct class.

Preliminary results show that when using all ASTM classes, other than miscellaneous and oxygenate classes, most of the samples classified using SIMCA were correct, or partially correct, with very few samples being incorrectly assigned. A partially correct sample was assigned to multiple classes that included the correct class, but also included incorrect assignment(s). For discriminant analysis methods, a two-class multi-step classification scheme was used.[1] The optimal QDA model gave correct classification rates greater than 90% for all steps; the optimal LDA model's correct classification rates were greater than 75% for all steps.

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Fire Debris, Chemometrics, Error Rates

A177 Utilizing GCxGC/TOF-MS to Improve the Data Quality for the Analysis of Fire Debris

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After attending this presentation, attendees will understand the ability of Comprehensive Gas Chromatography – Time-of-Flight Mass Spectrometry (GCxGC – TOFMS) to differentiate between gasoline samples as well as identify marker compounds that can help aid in arson investigations. The attendees will also recognize the difficulties associated with an arson investigation, the current extraction methodologies, and the reason a more sensitive and selective technique may be beneficial. The attendees will also be introduced to the use of Principal Component Analysis (PCA) for the evaluation of the site samples.

This presentation will impact the forensic science community by developing a technique that can allow for the differentiation of fresh and burnt gas samples and identifying marker compounds that indicate combusted gasoline versus gasoline that has been environmentally weathered.

Arsons can be particularly difficult crimes to investigate, due to the nature of the fire, which generally destroys most of the material evidence. Due to the lack of material evidence at the scene, the analysis of volatiles that may still be present from the use of common materials for fire initiation is typically evaluated. These volatiles, left from the fuel source used at the scene, may provide the evidence needed to connect an arsonist with their crime, and also determine the type of accelerant used. Historically, specific sources of accelerant were not able to be determined, in part due to the lack of selectivity of the analytical methods which have been previously employed.

The extraction methods utilized for these volatiles include gas headspace (under static or dynamic conditions), Solid Phase Micro-

extraction (SPME), and activated carbon strips with carbon disulfide. The most common extraction method, accepted for fire debris analysis and arson investigations, is headspace extraction of the fire debris with carbon strips followed by the extraction of the carbon strip with carbon disulfide. Appropriate instrumental techniques for the analysis of fire debris and ignitable liquid residues include GC/FID, GC/MS and/or GC/MS (SIM), and GCxGC/TOF-MS. The lack of selectivity of GC/FID has resulted in the more common use of GC/MS and/or GC/MS (SIM) because of the ability to evaluate the chromatogram for specific mass-to-charge ions, allowing for interpretation based upon compound classes. More recently, GCxGC has been considered for use in this application because of its ability to further separate compounds prior to detection. The resulting ability of the chromatograph to separate a hydrocarbon mixture based upon its compound classes (aliphatic, branched aliphatic, aromatic, etc.) enables the user of the data to quickly determine and describe the composition of the potential accelerant. Additionally this technique, due to its ability to provide higher data density, may allow for the identification of marker compounds to link the fuel with specific sources, and differentiate between gasoline used as an accelerant versus gasoline that has merely evaporated (weathered) as a result of normal degradation.

A case study from an active arson investigation of a forest fire utilized GCxGC/TOF-MS will be presented The technique's ability to identify the differences between un-weathered and weathered samples as well as pre- versus post-burn samples will be highlighted. Due to the environmental location of the fire, the differentiation between combusted gasoline and environmentally-weathered gasoline will also be noted. Statistical analysis was used to assist with the data sets and provide a convenient way to observe the similarities and differences between the samples.

GCxGC/TOF-MS, Fire Debris, Investigation

A178 A Comprehensive Study of Weathering and Microbial Degradation of Ignitable Liquids

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After attending this presentation, attendees will understand the concepts of weathering and microbial degradation of ignitable liquids from all ASTM classes.

This presentation will impact the forensic science community by highlighting the major trends in bacterial degradation of various examples of ignitable liquids from each ASTM class.

An organic-rich substrate such as soil is an excellent source of carbon for bacteria. Since ignitable liquids are comprised of various hydrocarbons, soil bacteria can also utilize these fuels as a carbon source. Previous work completed in our laboratory on the biodegradation of ignitable liquids has shown a significant loss of normal alkanes in the range of C_9 to C_{16} as well as lower substituted aromatic compounds. Branched alkanes appear to be more resistant to degradation than normal alkanes. In addition, the degree of degradation was positively correlated to the length of the alkyl chain on the mono-substituted alkylbenzenes. Also, the position of the alkyl branches plays a significant role in the ability of the bacteria to metabolize the alkylbenzenes. This can be problematic for fire debris analysis as samples may sit for many weeks before they are analyzed due to case backlog. As a result, selective loss of key components due to bacterial metabolism can make identifying and classifying ignitable liquid residues by their chemical composition and boiling point range very difficult. Of particular interest in this study is the monitoring weathering and degradation of various ignitable liquids in all ASTM classes in order to expand an existing database that can be used by fire debris analysts as a tool in the identification of ignitable liquids.

Weathering is the evaporation of the more volatile compounds of an ignitable liquid resulting in a greater concentration of the less volatile compounds. Weathering of the ignitable liquid is typical in fire debris due to the heat of the fire. The effect of ignitable liquid weathering on the chromatographic profile is the loss or reduction in intensity of the peaks at earlier retention times and an increase in intensity of the peaks at later retention times. The altered chromatographic profile of the weathered ignitable liquid residue makes comparisons to un-weathered reference ignitable liquids more complicated.

For degradation experiments, 20µL of the ignitable liquid was spiked onto 100g of potting soil and allowed to age for zero, seven, fourteen, and twenty-one days. The samples were then analyzed using passive headspace adsorption followed by solvent desorption, and then analyzed by GC/MS. Weathering of the ignitable liquids was performed by volume reduction of 10mL of ignitable liquid to 7.5, 5.0, 2.5, 1.0, and 0.5 ml corresponding to 25%, 50%, 75%, 90%, and 95% weathered, respectively. The ignitable liquids were heated in a dry bath under a gentle flow of nitrogen. At each evaporation point, a 20µL aliquot of the ignitable liquid was diluted into 1mL of carbon disulfide for GC/MS analysis.

This study will show that gasoline and petroleum distillates, which predominantly contain aromatic and n-alkane hydrocarbons respectively, suffer significantly from microbial degradation in just a few days while naphthenic-paraffinic liquids, which contain branched and cyclic alkanes, generally suffer the least microbial degradation. Oxygenated liquids also are significantly degraded; however, recovery of the oxygen-containing compounds in these liquids may present a challenge. Isoparaffinic liquids also suffer from microbial degradation. When comparing branched alkanes, long chained alkanes with one alkyl substituent are less resistant to microbial degradation than alkanes with multiple alkyl substituents. The changes in the chromatographic profile of various ignitable liquids described herein are a result of the selectivity of the bacteria to metabolize the hydrocarbons found in these liquids, which is solely based on the chemical structure of the compound. Weathering is a process that is based on the boiling point of the compound. In weathering, compounds with a lower boiling point will be lost preferentially; whereas, in microbial degradation, longer chain and less branched compounds are lost preferentially.

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Fire Debris, Ignitable Liquid, Degradation

A179 Principal Components Analysis and Hierarchical Cluster Analysis for the Identification of Ignitable Liquids in Simulated Fire Debris

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After attending this presentation, attendees will have an understanding of how multivariate statistical procedures can be used to aid the identification of ignitable liquids in fire debris, demonstrating the use of Principal Components Analysis (PCA) and Hierarchical Cluster Analysis (HCA) for the association of simulated fire debris samples to appropriate ignitable liquid standards.

This presentation will impact the forensic science community by presenting a more objective method for the analysis of fire debris samples. The application of PCA and HCA in this manner provides a statistical basis for the comparison of forensic evidence addressing concerns regarding the subjectivity of evidence comparisons that were raised in the 2009 Report published by the National Academy of Sciences.

Following analysis by gas chromatography-mass spectrometry (GC/MS), chromatograms obtained from fire debris samples are

compared to chromatograms of ignitable liquid standards to identify the presence of any ignitable liquid in the debris; however, several factors can complicate the chromatogram from the debris, making this comparison more challenging. Examples include the loss of volatile compounds, as well as the introduction of compounds that can be inherent to the debris itself or products of thermal degradation. As a result of these factors, the chromatogram from the fire debris sample may be visually dissimilar from the chromatogram of the corresponding liquid standard.

The purpose of this research was to investigate the use of multivariate statistical procedures to compare chromatograms from simulated fire debris to appropriate liquid standards, despite evaporation and the presence of matrix interference compounds and thermal degradation products. Three liquids were selected from each of three classes (naphthenic paraffinic, isoparaffinic, and alkane) as defined by ASTM International. Each liquid was evaporated to 0% and 50% by volume, then spiked onto a Kimwipe and extracted using a passive headspace procedure. A nylon carpet/carpet padding matrix was used in this research to represent a common household material. Samples of the matrix were initially burned for predefined times to determine an appropriate burn time that generated significant matrix interference compounds. To simulate fire debris, each liquid standard was spiked onto separate samples of the unburned matrix, which was subsequently burned for the appropriate time. These samples were then extracted using the passive headspace procedure, then analyzed by GC/MS.

Before analysis of the resulting chromatograms, it was necessary to perform data pretreatment procedures to minimize non-chemical sources of variance. Total ion chromatograms of the standards and debris samples were first smoothed to minimize instrument noise, thereby improving the signal-to-noise ratio. The chromatograms were then aligned to account for shifts in retention time, and finally normalized to account for slight differences in the injection volume.

The association of the simulated fire debris samples to their respective liquid standards was first evaluated using PCA. This statistical procedure is used to reduce the dimensionality of the data and identify sources of variance with the dataset. Results from PCA are shown in the form of scores plots and loadings plots. In the scores plot, chemically similar samples are positioned closely and distinctly from chemically different samples. The loadings plots are used to identify those compounds within the liquids contributing most to the variance described by the principal components. Principal components analysis was performed only on chromatograms of the liquid standards to demonstrate that the liquids could be differentiated based on liquid type and evaporation level. Then, the fire debris samples were projected onto the scores plot to investigate association to the corresponding liquid standard.

Hierarchical cluster analysis is used to assess the similarity of samples within a dataset, displaying results graphically in the form of a dendrogram. Samples that are clustered closely in the dendrogram are considered more similar. In this research, HCA was performed on the full dataset to investigate clustering, or association, of the fire debris samples with the appropriate liquid.

Using both PCA and HCA, it was possible to associate the debris to the type of liquid, despite evaporation and the presence of matrix interference compounds and thermal degradation products, from the carpet/carpet padding matrix. These procedures may provide an objective method that can be applied for the analysis of fire debris.

Fire Debris Analysis, Multivariate Stats, Chemometrics

A180 Professional Ethics and the Introduction of New or Novel Methodology

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After attending this presentation, attendees will have a better understanding of the ethical and scientific obligations in the introduction of evidence based on new or novel methodology.

This presentation will impact the forensic science community by providing a description of the differing ethical responsibilities of the various participants based upon differences in their professional duties.

The word "ethics" is derived from the Latin "*ethos*" which translates as "customary behavior." Webster defines ethics as: "the moral principles which determine the rightness or wrongness of particular acts or activities." Simply put, ethics deal with the commitment to do what is right, good, and proper.

The definition of ethics used for this presentation is from Oxford: "the science of human duty in its widest context." If "human" is replaced with "professional" this definition may be applied to any profession. By linking "ethics" with "duty" in a professional context, a basis is established for understanding how a specific conduct may be considered quite ethical for one profession yet patently unethical for another—their duties are different. Nowhere is this more apparent than with forensic science because of its close association with three professions—science, law and law enforcement.

For example, forensic science and law enforcement are distinct professions with complementary but differing duties. During an investigation, it is quite acceptable for the police investigator to rely on information obtained from a variety of sources and in myriad ways. Conversely, the conclusions of the forensic scientist must be based solely on the demonstrable scientific evidence developed. For science and law, the differences in duties are even more distinct. Scientists are expected to be totally objective, completely impartial, technically competent, and openly communicative; however, it is the duty of the attorney to aggressively represent the interests of his client whether it be "the people" or a defendant. It is the scientist's duty to describe the evidence as it actually is; it is the lawyers duty to describe it in the light most favourable to his client.

The attempt to introduce new or novel methodology can produce ethical challenges for each of the professions. These will vary depending on whether the evidence relates to a totally new form of evidence or whether it is simply a new application of an existing science. They also may be different at the investigative stage and at trial. The "one off" type of case will also be distinct from a new application to "regular" types of cases.

The onus is always on the forensic scientist to ensure that a new method is based on sound scientific principles, is well documented, produces reliable results, has its limitations and sample requirements clearly established, and has been peer reviewed. If it is intended to go beyond the "one off" case or the investigative stage, it should also have been published for appropriate broad professional review. In the absence of any of these, the scientist has not fulfilled his professional duty.

The law enforcement officer is required to ensure that the information developed by the scientist is relevant to the investigation, is based upon proper contextual information, and, if the information is to be used in decision-making, that it is used as intended and not distorted in any way.

The prosecutor has a duty to ensure that the method produces information that is relevant to the issue(s), that it meets the requirements for admission into evidence, and that it is presented thoroughly and effectively. The defense attorney has the duty to understand the method sufficiently that appropriate challenges may be made in the best interest of the defendant.

When new/novel methods are to be used, all the participants have specific duties and, thus, related professional ethical obligations.

Ethics, New Methodology, Professional Duty

A181 Enhancing Canine Performance Through Improved Training Materials and Adoption of International Best Practices (SWGDOG and ICODD)

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After attending this presentation, attendees will understand how improved training materials and the establishment of best practices for detection teams is improving interdiction efforts and courtroom

acceptance of dog alert evidence as well as the importance of creating an accreditation commission, the International Commission on Detector Dogs (ICODD). This presentation will impact the forensic science community by providing a better understanding of how the Scientific Working Group on Dogs and Orthogonal Detector Guidelines (SWGDOG) and accreditation through ICODD are improving the consistency and performance of deployed detector dog teams and their optimized combination with emerging electronic detectors.

This presentation will impact the forensic science community by providing the results of the latest studies identifying and quantifying odorants used by certified law enforcement and military detection canines and the development of odor mimics for use as field calibrants. A variety of factors can influence the measured performance of canine teams including the source of training materials, the containment system used, how the training materials are presented to detection teams, and how the teams were trained and maintained. The presentation will also describe the implementation of best practices developed by SWGDOG through the formation of ICODD. SWGDOG best practices have been developed by a membership of respected scientists, practitioners, and policy makers representing diverse backgrounds. SWGDOG has been cooperatively funded by the NIJ, FBI, DHS, and TWSG since 2005. This project was undertaken as a response to concerns coming from a variety of sectors, including law enforcement and homeland security, regarding the need to improve the performance, reliability, and courtroom defensibility of detector dog teams and their optimized combination with electronic detection devices.

The approval of each subcommittee best practice document takes a minimum of six months to complete including a two-month period of public comments. The 10 SWGDOG subcommittees are as follows: (1) unification of terminology; (2) general guidelines for training, certification, maintenance, and documentation; (3) selection of serviceable dogs and replacement systems; (4) kenneling, keeping, and health care; (5) selection and training of handlers and instructors; (6) procedures on presenting evidence in court; (7) research and technology; (8) substance dogs: agriculture, arson, drugs, explosives, human remains, contraband, pest, currency, and firearms; (9) scent dogs: non-specific human scent wilderness area search, location checks, article search, scent identification line-ups, live people in disaster environments, track trail people based on last known position, pre-scented canines aged trail, and live people in avalanche; and, (10) outreach and education.

The success of SWGDOG is dependent on the 55 SWGDOG members as well as the numerous external members within the working dog community who take the time to provide detailed commentary during the public comment stages. To date, there are *thirty-nine* approved guidelines within 436 pages of resources. SWGDOG is a catalyst in prioritizing research and development in both canine and orthogonal detector areas, in direct support of local law enforcement activities. The current success of SWGDOG is being manifested by a shift of some national canine organizations to adopt the approved SWGDOG best practice guidelines. Furthermore, the documents prepared thus far by SWGDOG members have been used to support the requirements of Homeland Security Presidential Directive -19 (HSPD-19), Combating Terrorist Use of Explosives within the United States.

Detector Dogs, Best Practices, SWGDOG

A182 Admissibility of Unconfirmed Accelerant-Detection Canine Alert

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After attending this presentation, attendees will understand current legal issues surrounding the admissibility of Accelerant-Detection Canine (ADC) alerts without laboratory analysis confirming the presence of ignitable liquids.

This presentation will impact the forensic science community by detailing how while a number of courts have followed the recommendations of the IAAI Forensic Science Committee and the NFPA Technical Committee on Fire Investigation and rejected the admissibility of expert testimony regarding unconfirmed alerts, others have nevertheless admitted this type of opinion evidence either as proof that an accelerant was present near the origin of the fire or that arson was the cause of the blaze. In arson cases, where the ultimate issue is whether a fire started accidentally or purposely, the jury could easily disregard the lack of laboratory confirmation and equate an uncorroborated alert with proof that the fire was aided by an accelerant. The probability of a wrongful conviction under those circumstances is not insignificant.

While the utility of ADCs in securing samples with a higher probability of laboratory confirmation may be generally accepted in the field of fire science investigation, the body of scholarly literature to date confirms that even the best-trained ADC cannot discriminate between accelerants and all possible background contaminants or byproducts of pyrolysis. As a result, the mere detection of traceable quantities of these substances has limited evidential value. Also, studies have shown they can falsely alert to areas of a fire scene that contain no trace evidence of ignitable liquids.

There is scant support for the proposition that a canine's olfactory sensitivity and specificity is more accurate than state-of-the-art GC/MS instruments employed in crime labs. This is why most scientists in the field conclude that an unconfirmed alert should be considered invalid, unreliable, and entitled to no weight. However, courts are not uniform in their approach to the question of whether these alerts are reliable.

The presentation will focus on the legal analysis on which courts rely in either admitting or rejecting expert testimony on unconfirmed alerts. This necessarily involves an examination of the issues of relevance, the prejudicial effect of this type of testimony and the tendency to confuse and mislead the jury, and foundation for expert opinions under the rules of evidence. Particular emphasis will be placed on the question of admissibility of such unconfirmed alerts under both *Frye*, *Daubert,* and *Kumho Tire* standards.

Canine, Accelerant, Arson

A183 Dog Scent Lineups: A Junk Science Injustice

Jeff Blackburn, JD, Innocence Project of Texas, 1511 Texas Ave, Lubbock, TX 79401*

After attending this presentation, attendees will understand the misuse of dog scent lineups in criminal cases.

This presentation will impact the forensic science community by pointing out the dangers in relying on unvalidated methodologies, often referred to as "junk science."

It is well known that dogs possess superior olfactory abilities compared to humans. Their ability to follow trails is well documented and accepted as an investigative tool. The use of dog scent lineups to associate a particular individual with a particular location is a more recent use of this tool — one which has found its way into criminal trials and resulted in wrongful convictions.

Under perfect conditions, with the careful application of controls to prevent undue influence on the canine by its handler, scent lineups may achieve an accuracy of 85%.[1] This presentation will focus on an individual who, without the benefit of any protocols or controls, claimed under oath that his canines were accurate more than 99% of the time.

Fort Bend County Sheriff's Deputy Sergeant Keith Pikett became very popular among Texas prosecutors because of his unique "ability" to associate defendants with crime scenes. He developed a down-home, folksy testimonial style and appeared at trials throughout Texas. Even after the prosecution bar became generally aware of some of Sgt. Pikett's shortcomings, they continued to call on him to help "solve" their cases.

Some of the testimony that prosecutors presented and judges allowed included the amazing claim that even though he did not keep detailed records, his dog "Clue" had only been wrong in one out of 1,659 lineups.[2] His dog "James Bond" had been wrong once out of 2,266 lineups, and his dog "Quincy" had only been proven wrong in three out of 2,831 lineups.[3,4] Pickett denied that he had any need of any formal training in scent lineups, he denied that he needed to follow any formal protocols, and he rejected the results of scientific studies.[5] Pickett

claimed his dogs could identify scent more than 10 years old, and could also identify scents from vehicles.[6,7]

In 2002, the case of *State vs. Winston* cemented Sgt. Pickett's status as the leading scent lineup expert in Texas. In this case, the 14th Court of Appeals held that Winston was a qualified expert.[8] Thereafter, Pickett was allowed to present his lineup testimony in Texas as "scientific evidence."

As is the case with many charlatans, Sgt. Pikett attempted to enhance his credibility by falsifying his credentials. Sgt. Pikett earned a Bachelor of Science degree in chemistry from the University of South Alabama in 1977 and a "Master of Sport Science" degree from the United States Sports Academy in 1984; however, at various times, he testified under oath that he held a Bachelor of Science degree in chemistry from Syracuse University and a Masters degree in chemistry from the University of Houston. Both institutions' registrars stated that they had never heard of the man.

As is the case with many instances of "expert" witness misconduct, there were no serious consequences for Sgt. Pickett. He was never reprimanded, never disciplined, and certainly never charged with perjury. After the Innocence Project released a report detailing Sgt. Pickett's career, he "retired" in January 2010. Meanwhile, prosecutors and police continue to use scent lineups.

References:

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7. Deposition testimony at 74-75 and 141, Buchanan v. city of Victoria *et al*., 6:2008cv00008 (S. D. Tex. Filed January 29, 2008)-Deposition taken on January 22, 2009.
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Canines, Scent Lineups, Junk Science

A184 Who Let the Dogs In? The Admissibility and Scope of Testimony of Dog Handlers

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The goal of this presentation is to explore the mistakes made in *U.S. vs. Hebshie* as examples of what defense counsel, law enforcement, and prosecutors can do in the future to avoid misuse of this valuable tool, also addressing these issues by reviewing both federal and state cases.

This presentation will impact the forensic science community by outlining the foundational requirements for allowing dog handlers to testify in court about the reactions of trained accelerant-detection dogs in detecting the presence of a fire accelerant in a criminal or civil arson cases, as well as the reactions of drug detection dogs.[1-3]

In 2006, James Hebshie was convicted and sentenced to 15 years in prison for arson and mail fraud for setting a fire in a commercial building where he worked.[4] In order to prove that the fire was arson, the Government elicited testimony from an accelerant-detection dog handler and a laboratory technician, who testified about the incendiary nature of the fire. Notwithstanding, the trial judges repeated suggestions to defense counsel to take time to consider the admissibility of this testimony, the defense counsel did not request a pre-trial hearing nor mount any meaningful challenge to the evidence at trial. In 2010, a motion was brought to vacate Mr. Hebshie's conviction, claiming that defense counsel's failure to mount a challenge to the "accelerant-detection dog" testimony constituted ineffective assistance of counsel.

Few people would challenge the principle that dogs generally possess an enhanced ability to detect certain oders such as food or specific sustances such as marijuana, heroin, cocaine, methamphetamines, and, perhaps, even fire accellerants. However, utilizing that ability to assist law enforcement personnel to uncover such substances requires specialized training in order to alert their handlers when they have detected such a substance. The alert may be a bark or scratching at the location, or it may be an obvious turn of the head or tail movment, or by just passively sitting at the location. Since all of these actions are part of a dogs usual activities, training the dog to perform in a specific manner to alert the handler that it has detected the specific substance for which it is trained differs with each dog. But without maintaining acurate data on how proficient a particular dog is in making positive alerts as opposed to false positive alerts to drugs or fire accelerants, there is no objective way to judge the reliability of that particular dog.

While certificates attesting to the dogs training should be an initial factor, continuous proficiency testing should be maintained and documented to ascertain a particular dogs error rate in order to determine the reliability of any particular dog. The use of dogs as an investigative tool should be used appropriately. When the government seeks to introduce evidence regarding a dog's role in the investigation, the question is not whether dogs can generally detect certain odors, but how acurate and proficient that specific dog is in detecting that specific odor. Courts as "gatekeepers" of evidence have an obligation not only to consider the admissibility of the evidence, but what limitations, if any, should be placed on the testimony of dog handlers.[5] Judge Nancy Gertner in the *Hebshie* case, stated "a certain patina attaches to an expert's testimony unlike any other witness; this is "science," a professionals judgment, the jury may think, and give more credence to the testimony than it may deserve."

This presentation will look at what judges and counsel should review in addressing the use of drug detection alert dogs and accelerant alert dogs as evidence in a trial and whether there are any differences. What are the parameters for the admissibility of this type of evidence? What underlying data should be required in order to allow the testimony of a dog handler in court? What can law enforcement do to improve the admissibility of such testimony? Finally, what obligation do the various participants in the criminal justice system have to assure that only reliable evidence is presented to the trier of fact?

While the judiciary has continued to struggle with how to address emerging technologies, courts are looking with a more critical eye toward the underlying principles, procedures, and methods employed by forensic specialists that were once assumed to be reliable and pondering whether to continue to admit such evidence into a trial without first knowing about its underlying validity.[6]

References:

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2. *Daubert v. Merrell Dow Pharmaceuticals,* 509 US 579 (1993).
3. *Kumho Tire v. Carmichael*, 526 US 137 (1999).
4. *United States v. Hebshie*, 754 F. Supp. 2d. 89 (D. Mass 2010) quoting*, United States v. Hines*, 55 F. Supp. 2d 62, 64 (D. Mass. 1999).
5. *Florida v. Jardines,* ___Sup. Ct.___(2012).
6. *Florida v. Harris,* ___Sup. Ct ___(2012).

Dog Handler, Accelerant Detection, Admissibility

A185 Forensic Attribution of Matchsticks Using Integrated Analysis With Morphological Examination, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDS), and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Ciaran F. Phillip, BS, and Christopher J. Ehrhardt, PhD, Virginia Commonwealth Univ, Dept of Forensic Science, 1015 Floyd Ave, Richmond, VA 23284*

After this presentation, attendees will be able to recognize the unique physical and chemical features present in match heads with different functions and match heads with the same function but originating from different manufacturers; also learning how these features can be incorporated into a multivariate signature that can be used to identify the specific type and commercial brand of an unknown match.

This presentation will impact the forensic science community by introducing a scientifically and statistically robust analytical scheme for collecting forensically relevant information from question matchsticks. Such forensic signatures can provide investigative leads in criminal cases and better illustrate the association between question and known matches to a jury.

Because matches are designed for quick fire starting, they are quite amenable to criminal activity and are often encountered in arson and bombing cases. Different functional types of matches display physical and chemical differences in both burned and unburned match heads that are specific to that match type. Furthermore, previous research has shown that there are chemical features in match heads that are not shared by matches from different brands; however, no project before this has attempted to combine the physical and chemical characteristics of both spent and unspent match heads in order to create multivariate signatures to be used for functional and commercial brand classifications.

There are three main functional classes of matchsticks: safety, strike-anywhere, and waterproof. Safety matches are the most pervasive and widely used type of match. They often find use in kitchens, places of worship, and most other instances where safe fire starting is required. The components for igniting these matches are separated into a match head and a specially designed striking surface. In contrast, strike-anywhere matches are not common and are generally used by outdoor enthusiasts as a reliable method for starting fire. They have all ignition components in the match head and can be lit on any suitably rough surface. Waterproof matches are safety matches (can only be lit when struck on a specially designed surface) that have been coated with a water-resistant, yet flammable, polymer binder. Like strike-anywhere matches, waterproof matches are used as outdoor survival tools by campers, hikers, and even the military.

For this project, four brands of safety, two brands of strike-anywhere, and four brands of waterproof matches were used. Safety match brands included: Diamond™ Strike On Box, Diamond™ Deluxe Matchbooks, Industrial Revolution/UCO Long-Burn, and HomArt® Fancy Fish Superior Quality. Diamond™ and Redbird were the strike-anywhere match brands, and Coleman®, REI® Stormproof, Proforce Equipment, and Coghlan were the brands of waterproof match. Matches were analyzed using an analytical scheme of stereomicroscopy for documenting visual characteristics, Fourier Transform Infrared Spectroscopy (FTIR) to identify any binder coating present, scanning electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDS) to determine elemental composition, and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) to measure multi-elemental concentration profiles.

Results showed that matches do have distinct physical characteristics that are unique for particular commercial brands and types. Match head color, texture, structure, and metrics were particularly excellent markers for indicating a match's functionality and commercial brand. Chemical analyses revealed that different commercial brands of waterproof match have different polymer binders including nitrocellulose (Coleman® and Coghlan), alkyd (REI Stromproof), and shellac (Proforce Equipment). No polymer binder was identified in safety or strike-anywhere matches. Elemental composition was found to be similar among all match types and brands with the majority of the samples containing silicon, potassium, chlorine, magnesium, titanium, iron, zinc, chromium, lead, thorium, and uranium. However, concentrations vary significantly between brands. For example, average ^{70}Zn concentration was 247.7ng·mL^{-1} and 60.2ng·mL^{-1} in Diamond and Redbird strike-anywhere matches respectively. Similar differences were seen for ^{24}Mg, ^{57}Fe, Pb (all isotopes), ^{232}Th, and ^{238}U. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) performed on the multivariate data set produced distinct clades for waterproof match brands, while safety and strike-anywhere match brands were distributed throughout the cladogram. Taken together, these results indicate that robust signatures exist for functional classes of matchsticks and individual commercial brands. Implementation of this analytical scheme with multivariate statistical analysis will increase the evidential value of question matches providing leads for investigators and strengthening conclusions of consistency between question and known evidence.

Matchstick Typing, ICP-MS, Multivariate

A186 Evaluation of Lip Cosmetics Using Raman Spectroscopy

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After attending this presentation, attendees will be informed about the possibility of using Raman spectroscopy in the analytical scheme for lip cosmetics.

This presentation will impact the forensic science community by suggesting a non-destructive, rapid method for evaluation of lip cosmetics that can provide significant discriminatory information for comparisons.

Because of the ease of transfer and prevalence of use, lip cosmetics are often encountered on crime scenes. The vast array of different manufacturers, product types, and colors make them a potentially powerful source of associative evidence. However, many current analytical schemes need components of lip cosmetics to be extracted and analyzed separately. For example, dye components may be extracted and analyzed with High Performance Liquid Chromatography (HPLC) or Thin Layer Chromatography (TLC), while oils and waxes may be analyzed using Gas Chromatograph/Mass Spectrometry (GC/MS). Raman spectroscopy can give a more complete look at chemical composition without need for extraction procedure, which reduces both handling time for the sample and risk of contamination.

In this study 80 lipsticks, 34 lip glosses, and 17 lip balms were obtained from donations and evaluated using Raman spectroscopy at an excitation of both 532 and 780nm. Pure samples were analyzed as smears on aluminum foil. Intra-product studies were first conducted to determine the effect of dye combinations when the base composition was unchanged. Four common colors, red, brown, purple, and purple-brown were selected from the lipsticks and grouped with ten similarly colored products from different manufacturers for comparison. Any two lipsticks outside these groupings with visually identical colors were also compared. The possibility of examining cosmetic samples directly from a substrate (such as cotton swabs, colored fabrics, and automobile panels) was also evaluated. For substrate studies, two identically colored lipsticks were selected and applied to the desired surface and examined without extraction. The ability to distinguish the lipstick from the substrate as well as the ability to differentiate between lipstick samples was assessed. Spectra were compared using visual overlays.

While fluorescence severely limited the analysis by the 532nm source, the 780nm source provided useful spectra from all analyzed samples. Within a brand, colors from the same product line could be distinguished from one another using Raman spectroscopy alone. This

was true even for colors that were very close to one another, indicating that dye components contribute significantly to the Raman spectra. It was also possible to distinguish between the same colors from different products within the same manufacturer.

In inter-brand comparison of similarly colored samples, the lipstick samples could all be discriminated using only the Raman spectra. Many similarities were attributed to dyes used, but there was enough variation between dye formulations and other chemical components to provide unique spectra. Lip glosses and lip balms showed less variation within the same product, mostly likely due to less contribution from pigmentation. It was also possible to distinguish the lipstick signal from the substrates examined. However, there was detail lost from the pure spectra. Despite this, it was still possible to use the spectra as a source of discriminatory information.

Instances where spectra could not be differentiated were limited to lip glosses and lip balms of different colors from the same product type and manufacturer. No lip glosses or lip balms had the same spectra as a lipstick. Variation in chemical composition of lip cosmetics provides significant discriminatory power and opens the possibility of creating a database that catalogues Raman spectra of lip cosmetics.

Lip Cosmetics, Lipstick, Raman Spectroscopy

A187 Differentiation of Color Toners on Paper Using Raman Analysis and Chemometrics

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After attending this presentation, attendees will understand the advantages of using Raman spectroscopy in questioned document casework to further differentiate photocopier and laser printer toner cartridges. In addition, attendees will gain an understanding of multivariate statistics as they apply to this particular type of evidence.

This presentation will impact the forensic community by demonstrating a spectroscopic method of analysis that is not only quick and non-destructive with no sample preparation, but is not plagued by the interference of paper, also addressing the recommendations set forth in the National Academy of Sciences' report. For example, there is a need for continued research in many areas of forensic science that could contain a statistical component. In particular, visual examination and comparison of two spectra cannot always detect subtle differences. However, the same spectra could be objectively compared through the application of multivariate statistical analysis.

Toner analysis has become an area of increased interest due to the wide availability of laser printers and photocopiers. Toners contain a number of components, including, but not limited to, polymer resins, dyes/pigments, surfactants, and charge control agents. The formulation of these toners, granule size, and melting point can vary between manufactures and machines. In addition, toner is most often encountered on paper in questioned document analysis. Because of this, it is important to develop methods that limit the interference of paper without damaging or destroying the document. Previous research using Fourier Transform Infrared Spectroscopy (FTIR) has differentiated toners based on their polymer resin components. Developing a method to differentiate toners based on their other components is important for increasing the discriminating power of toner analysis.

Raman spectroscopy is a popular tool for the chemical analysis of pigmented samples. The technique involves measuring the vibrational modes of a molecule in the form of inelastic scattering of light after being subjected to a monochromatic light source, such as a laser. The use of a microscope provides a very high level of spatial resolution and depth discrimination. However, the interference from carbon black and iron oxide contained within black toner make it a problematic sample for analysis. As a result, cyan, yellow, and magenta toners were the focus for this study. Analyses were performed using a dispersive micro-Raman spectrometer equipped with a 785nm diode laser, a CCD detector, and an objective at 20X magnification. Three different methods were developed for cyan, yellow, and magenta toners on paper, respectively, to optimize results. One hundred samples of each color toner were collected. Cyan toner samples were subjected to 10% laser power with an exposure time of nine seconds for 15 scans. Yellow toner samples were subjected to 100% laser power with an exposure time of 10 seconds for 15 scans. Magenta toner samples were subjected to 25% laser power with an exposure time of 10 seconds for 30 scans. Due to the increased paper interference in the yellow and magenta toner samples, spectra of the paper for each sample were collected and spectral subtraction was performed. The data collected from these analyses was then processed using a combination of statistical procedures, including Principal Component Analysis (PCA), Agglomerative Hierarchal Clustering (AHC), and Discriminative Analysis (DA). The conclusions drawn from this study were used to form a classification scheme for each colored toner.

Raman Spectroscopy, Color Toners, Chemometrics

A188 Forensic Examination of Artificial Sweeteners

Vincent J. Desiderio, MS, Andrew M. Bowen, MS*, and Robert B. Moberley, MS, U.S. Postal Inspection Service, National Forensic Laboratory, 22433 Randolph Dr, Dulles, VA 20104-1000*

After attending this presentation, attendees will: (1) become aware of the analytical challenges posed by the forensic examination of artificial sweeteners, occasionally submitted to the laboratory as unknown materials in "white powder" cases; and, (2) understand the advantages and disadvantages of various instrumental approaches to characterizing and identifying these products when they are encountered as evidence.

This presentation will impact the forensic science community by providing recommended analytical approaches for the most effective characterization and identification of artificial sweeteners. This will improve their ability to examine this type of evidence in the event that they encounter it in their laboratories.

After the 2001 Amerithrax case that involved mailed letters containing anthrax spores, "white powder" incidents became relatively common events in the news. Law enforcement officials focused significant resources on the development of capabilities for responding to and identifying suspicious white powders encountered in public spaces. These efforts resulted in the creation of a variety of first-response teams affiliated with police departments, firefighters, National Guard, and other federal law enforcement groups. After an initial assessment of the threat posed by the white powder (often utilizing portable instrumentation or mobile laboratories designed to rule out specific weapons of mass destruction), the material is typically collected for submission to a laboratory for more specific identification. These powders, therefore, have the potential to end up in forensic laboratories as evidence. Another result of the widely publicized anthrax case is the relatively common occurrence of copycat individuals who include white powders in threatening communications often mailed to victims. Many of these letters are submitted as evidence to the National Forensic Laboratory of the U.S. Postal Inspection Service, or to other forensic laboratories around the country. As a result, there is a continuing need for forensic laboratories to be well prepared to identify unknown materials in general, and white powders in particular.

Although some of these powders are hazardous materials, the vast majority end up being common household products or commercially available chemicals. It is the job of the laboratory to identify these materials and, in many cases, to subsequently compare them with products obtained from the residence or workplace of a suspect. The majority of these commercial products are fairly straightforward to characterize and identify; however, some materials pose analytical challenges for the typical forensic laboratory. Several of the commonly used artificial sweeteners are so sweet that they are used in very small quantities in the final consumer product. The vast majority of the powder in these products consists of fillers (typically D-glucose). The fact that the sweetening agent is present in such small quantities can make it

challenging to unambiguously determine the identity of the product or compare it to similar products submitted to the laboratory. The National Forensic Laboratory has received artificial sweeteners as evidence associated with threatening letters in the past, prompting the laboratory to acquire and characterize standard artificial sweeteners using a wide variety of analytical techniques, including stereomicroscopy, polarized light microscopy, Fourier Transform-infrared spectroscopy, scanning electron microscopy, energy dispersive spectroscopy, X-ray diffraction, Raman spectroscopy, and gas chromatography-mass spectrometry.

This presentation will introduce the audience to the commonly encountered sweetening agents and discuss their occurrence in commercial products. The results obtained during characterization of several of the most common artificial sweeteners will also be discussed. The advantages and disadvantages of the various instrumental techniques for the purpose of identifying these sweetening agents will be considered. Suggestions will be made to the audience related to appropriate analytical approaches to the examination of suspected artificial sweeteners. The work presented here will provide the foundation for the future development of an analytical scheme for characterizing and identifying all of the common artificial sweetening agents.

Sweeteners, White Powders, Chemical Unknowns

A189 The Microscopic Study of the Statistical Significance of Household Dust Specimens

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After attending this presentation, attendees will understand some of the tenets behind principal component analysis, support vector machines, and partial least squares discriminant analysis in order to build statistical models of the composition profiles in the study of household dust specimens.

This presentation will impact the forensic science community by establishing the statistical significance of household dust specimens in trace evidence casework; its high confidence level of 99% and error rate of less than 1% reported will go a long way in re-establishing trace evidential materials as a powerful form of scientific evidence.

This paper continues the discussion and study of the statistical significance of household dust specimens presented at the 2011 AAFS Annual Scientific Meeting in Chicago. Additional results of the original household dust specimens, as well as new results for household dust specimens obtained from homes, businesses, and apartments encompassing the New York City Tri-State region, and from other areas in the United States are presented.

The original procedure which began with a preliminary visual and stereomicroscopic examination of each dust specimen has been expanded in order to include a more diverse array of particulate materials. The contents of each new dust specimen are sorted with the aid of a stereoscopic microscope into groups of similar-looking: hairs, fibers, and particulate materials depending on the composition of each dust specimen's composition. In addition, aggregate groupings of fibrous and particulate materials are teased out of each dust specimen and examined. Each aggregate group is further subdivided into smaller subsets based on their macroscopic physical appearance, primarily color and morphology. Each fibrous and/or particulate subset is mounted on a 7.5cm x 5cm microscope slide in Cargille® 1.540 High Dispersion (HD) refractive index oil. Finally, each fibrous and/or particulate subset aliquot is characterized and identified utilizing stereo and polarized light microscopy. Three aliquots were examined from each household dust specimen. Particulates too large to be mounted for PLM examination were studied with other forms of analytical instrumentation, i.e., Fourier Transform Infrared Spectroscopy, X-ray Fluorescence, and X-ray Diffraction.

The resulting information for each new specimen and its mounted subset was collected on a revised dust tabulation data sheet specifically designed for this study. The newly acquired data and the data collected in the prior study were combined and resubjected to principal component analysis, support vector machines, and partial least squares discriminant analysis in order to build statistical models of the composition profiles. These statistical models were then subjected to a test set of randomly selected unlabeled dust samples in order to compute estimates of misidentification rates (error rates). The methods used to compute these error rates were hold-one-out cross validation and bootstrapping.

A number of interesting trends, such as the ubiquitous occurrence of certain types of natural fibers, the recent occurrence of a wider range of species of natural fibers, the appearance of microfibers, the appearance of faux fibers, the proliferation of green fibers, and the expansion of more diverse mixtures of synthetic fiber types as well as the frequent occurrence of several classes of biological and particulate materials, are extensively discussed. Recommendations regarding the use and value of these ubiquitous materials in establishing whether a contact transfer occurred based on their presence are statistically evaluated and discussed at length.

In particular, the building of sound scientifically acceptable statistical models that meet the challenges of the NAS Report on Forensic Sciences, and establish high confidences, levels and accurate error rates for the microscopic examination of forensic dust specimens were primary goals in this study.

Statistical Significance, Household Dust, Trace Evidence

A190 Forensic Discrimination of Copper Items Using Trace Element Concentrations

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After attending this presentation, attendees will understand the general principles of source discrimination, method development for determination of trace element concentrations using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), and the statistical comparison of trace element concentrations in copper from different sources for discrimination among and association between sources.

This presentation will impact the forensic science community by potentially providing a new means to associate crime scene and suspect-linked copper items based on their trace element concentration profiles.

Copper can be encountered in criminal cases such as theft (as raw material from mines and as wiring or piping from construction sites and homes) and bombings (wiring in explosive devices). At present, the only means of reliably associating copper items is on the basis of fabrication marks, which may not be possible in all cases depending on the copper item. The goal of this research is to assess the potential of using the trace element concentrations of a copper item from a crime scene to either associate or eliminate as a source a copper item associated with a suspect.

The conductivity of copper is dependent on its purity (i.e., concentration of trace element contaminants). Rapid techniques such as spark source optical emission spectroscopy and X-ray fluorescence spectroscopy are commonly used in production laboratories to determine trace element impurities in copper. However, quantitation limits for these techniques are close to or higher than the analyte concentrations in high purity copper. Due to the requirement of lower detection limits, an alternative method based on ICP-MS instrumentation was explored.

Concentrations of trace elements in copper were initially determined using solution ICP-MS in NIST standard reference materials for method validation. It was determined that a microwave digestion step after initial dissolution of the copper samples in concentrated nitric acid was required to achieve quantitative recovery of all analyte elements. The standard reference materials were also used to construct a matrix-matched calibration curve, which was compared with non-matrix-matched calibration. To increase the number of elements available for source discrimination, noncertified elements present in the

standard reference materials were identified as present using solution and laser ablation ICP-MS, although the accuracy of the method for these elements cannot be confirmed. Relevant figures of merit determined include method detection limits based on 10 replicate blanks that were carried through the sample preparation process, matrix effects due to high concentrations of copper, method accuracy by comparison of measured and certified analyte element concentrations, and reproducibility of the sample preparation and measured concentrations.

To discriminate between copper items, the variation in trace element concentrations within a "source" (e.g., from different parts of the copper item and/or from the same production batch) must be small compared to the variability in trace element concentrations between production batches from the same manufacturer, items produced by different manufacturers, and geographic location of raw material mining. Samples were collected to represent copper from different geographic regions, refining operations, and production procedures used to produce specific types of copper items. Statistical techniques are used to identify elements that contribute significantly to the variation within and between samples, and to group samples based on their trace element concentration profiles. Statistical analysis of the trace element concentration profiles could potentially provide a link between the copper item recovered from the crime scene and an item associated with a suspect, or exclude a suspect from further investigation.

Source Discriminatio, Trace Elements, Copper

A191 Duct Tape Sourcing Examinations: Developing Investigative Leads Using Multiple Resources

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After attending this presentation, attendees will have knowledge of the methodology used to source duct tape bindings using a combination of factors that can provide highly discriminating investigative lead information. These factors include physical characteristics, instrumental analyses such as X-ray Diffraction (XRD) and Pyrolysis-Gas Chromatography/Mass Spectrometry (Py/GC/MS), a duct tape reference collection and database, and industry contacts. Results demonstrate that the combination of these factors can provide highly discriminating investigative lead information.

This presentation will impact the forensic science community by disseminating knowledge regarding discrimination achieved through XRD and Py/GC/MS analyses, two techniques not commonly associated with duct tape analyses. Further, the use of a reference collection and searchable database provided an efficient means of culling large-scale manufacturers and distributors from the list of possible tape brands.

The case example presented will describe how the FBI Laboratory was able to develop investigative lead information in a homicide case. When investigators arrived at the crime scene, they observed a deceased victim and a witness loosely bound with duct tape. The witness stated that both persons were victims of a robbery. Local law enforcement requested forensic examinations to further their investigation, including sourcing of the tape.

The bindings were examined in order to document physical characteristics such as the backing construction, adhesive color, width, scrim count and construction, and the backing and overall tape thicknesses. Chemical analyses of the adhesive and backing components of the duct tape were conducted via Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM/EDS), XRD, and Py-GC/MS. From the FTIR results of the adhesive, talc was readily observed, but the rubber and tackifying resin composition were difficult to interpret. XRD results revealed contaminants, tremolite and phlogopite, not previously identified in duct tape adhesives analyzed in the FBI Laboratory, suggesting the tape was not a commonly available product. Tremolite is a form of asbestos and phlogopite is a sheet aluminosilicate that falls into the mica class; both can be found as contaminants in common filler and extender pigments such as talc and dolomite. Py-GC/MS was used to elucidate that the adhesive formulation was styrene-butadiene-styrene (SBS) when FTIR results did not adequately distinguish between SBS or the use of a tackifying resin in a styrene-isoprene-styrene (SIS) formulation. The distinction was important for sourcing purposes given that most manufacturers use SIS or isoprene alone.

Using the National Forensic Tape File (NFTF) reference collection and database, industry contacts, and the Internet, a single manufacturer was able to be identified, and from it, a specific tape product was targeted as the source of the bindings. When sales data for this product was evaluated for the state where the crime occurred as well as its three neighboring states, it was found that only one distributor bought the product and in turn sold it to eight industries: mostly mid-size manufacturers of consumables (e.g., paper products), durable goods (e.g., furniture, storage tankers, food services), and two locations of a funeral service provider.

This case highlights the utility of employing a variety of analytical and reference-based resources to source manmade, mass-produced materials. It is also unusual in that the chemical analyses proved to be more discriminating than the totality of the physical characteristics of the tape due to the raw material choices of the manufacturer, both the specified choice of SBS and the filler with the readily observed impurities. Compelling investigative lead information was reported to the contributor using the described resources, most of which are readily available or can be developed by any laboratory system interested in conducting duct tape sourcing examinations.

Duct Tape Sourcing, Instrumental Analysis, Reference Collection

A192 Operation Invisible Dead: A Blind Study of Soil Identification Using a Microbiological Database

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After attending this presentation, attendees will understand more thoroughly the application of soil microbial community profiling and the potential use of a soil database to identify microbiological soil evidence.

This presentation will impact the forensic science community by exploring the use of Capillary Electrophoresis-Single Strand Conformation Polymorphism (CE-SSCP) to identify soil samples using a soil microbiological database.

Soils have been used as trace evidence for well over a century. As technology has advanced, so has the value of soil evidence. Most recently, soil microbial community profiles have been used to provide an accurate discriminating factor between soil samples of known and unknown origin. The use of microbiological physical evidence can detect the uniqueness or similarity of the soil evidence when compared to the collected reference soil samples from the crime scene. The current literature contains several papers that test the accuracy and limitations of these methods; however, no one has yet done a case study on how well soil microbial community identification works with a database. Thus, a blind study was set up with a database to test the sensitivity and reliability of the soil microbial evidence.

The blind study was set up where a "criminal" dug a shallow grave using a shovel. Upon completion of the shallow grave, the "criminal" then placed the shovel and one shoe into individual paper bags for evidence collection. The other shoe was worn back to a place of residence, and then placed in a paper evidence bag. The shovel and two shoes were allowed to air dry at room temperature for 48 hours. The soil was collected from these objects by a different undergraduate researcher. This analyst conducted all sampling and handling without knowledge of the location of the grave. DNA from the soil was extracted

using a commercially available DNA extraction kit. Amplification of the extracted DNA was done with fluorescently labeled primers and a proofreading enzyme for the V3 region of the 16S ribosomal DNA. Using CE-SSCP the soil microbial community was characterized. This method yields electropherograms of the microbial community DNA specific to the bacteria present in the soil sample. The profiles of the soil microbial community were compared to a database of four soil plots in southeastern Nebraska over three different seasons. The database was made from a previous research project and holds 72 profiles. The samples in the database were processed with DNA extracted within 24 hours of removal of soil from the ground at a depth of 0cm to 5cm. DNA extraction, Polymerase Chain Reaction, and CE-SSCP for the samples in the database were all processed the same way as the samples from this study. It is understood that this is a relatively small database, but it can help to initiate the usefulness of a soil microbiological database.

The database has been made and the samples have been processed via CE-SSCP. The results of the study are two-fold. When visually examining the soil microbial electropherograms from CE-SSCP, the location of the grave was correctly identified. However, to ensure a reliable outcome is obtained, statistical analysis must be conducted. R software will be used to statistically compare and contrast the samples of interest to the database.

Soil, CE-SSCP, Microbial DNA

A193 An Unusual Tool/Fracture Mark Case to a Smokestack From a 19th-Century Steam Engine

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The goals of this presentation are to educate about examination of tool marks and the use of fracture matches to determine the common origin of a portion of a 19th-century cast-iron smokestack and also learn about the theft of antiquities from federally protected archeological sites and how the Antiquities Act of 1906 and the Archaeological Resources Protection Act of 1979 apply to persons accused of theft, and also to educate the participants on the history and processes of gold mining in Kern County, California.

This presentation will impact the forensic science community by helping them understand the process of forensic examinations of antiquities illegally harvested from archaeological sites.

The theft of antiquities from federally designated archeological sites is a growing problem in the United States. The price of iron as well as other metals such as copper, silver, and gold in the recycled metals market has steadily increased, thus making the harvesting of metal-bearing materials from abandoned sites economically attractive. The unfortunate effect of this phenomenon is the vandalism, destruction, and theft of antiquities from unprotected federal lands. The Antiquities Act of 1906 signed by President Theodore Roosevelt and the Archeological Resources Protection Act (ARPA) of 1979 were enacted to protect antiquities from being obliterated, removed, or destroyed from federal and Native American lands.

In the summer of 2011, the Bureau of Land Management (BLM) submitted evidence to the Kern Regional Crime Laboratory (KRCL) items that had been illegally harvested from an abandoned gold mine designated as an archeological protected site. These evidence items included the following: a flattened riveted cast-iron section of 19th-century smokestack seized from the suspect, a concrete cutting saw with circular metal cutting wheel seized from the suspect, a rounded section of a cast-iron 19th-century smokestack recovered from the mine site, and a circular metal cutting wheel recovered from the mine site. BLM requested the laboratory to determine if the flattened smokestack seized from the suspect's vehicle was at one time part of the smokestack remnant recovered from the mine site.

An examination of the flattened section seized from the suspect and the remnant from the mine site was conducted using standard comparison methodology and recorded using digital photography. The process of restoring the flattened section of smokestack, the comparison of cut marks and fracture mark analysis, the legal implications of ARPA, and a brief history of gold mining and its processes in Kern County California will be discussed.

The process of examination included:

1. Visually determining if the two items are compatible.
2. Reshaping and reforming the unknown items that an attempted alignment could be accomplished.
3. Aligning the items to determine where common tool marks could be compared.
4. Searching for fracture matches when conventional tool marks comparisons were not feasible.
5. Identifying fracture matches to establish common source.

Conventional tool mark techniques were not used due to the nature of the cutting instrument, which was determined to be a worn-down grinding wheel on a gas-powered concrete saw. However, a fracture match was identified in an area were the grind cuts were incomplete. The fracture match was difficult because of the brittleness of the metal caused from years of oxidation and corrosion in the open-air environment.

In conclusion, fracture mark comparison is possible when conventional tool mark examination and identification techniques fail, working with decomposing and corroded metal has its challenges when attempting common source identifications, and 19th-century manufacturing techniques do allow for individualization.

Fracture Match, Tool Mark, ARPA

A194 Virtual Tool Mark Generation for Efficient Tool Mark Analysis

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After attending this presentation, attendees will be informed about ongoing research into a computer-aided method for tool markanalysis that uses virtual tool marks to predict the angle of a real tool mark. Attendees will learn about the basic procedure, scientific principles, and potential advantages of this new technique and see a preview of the current state of the software development. They will also have the opportunity to provide input on the direction and quality of the research and suggestions for future methods that could be developed.

This presentation will impact the forensic science community by demonstrating a statistical method that can not only strengthen the rigor of impression evidence analysis, but also increase its efficiency. In particular, the presentation will focus on an application of the statistical algorithm to estimate the tool-marking angle and twist of a real mark. This knowledge could prevent examiners from generating extra marks at unprofitable angles, saving time and reducing possible damage to evidence.

In 2009, a National Academy of Sciences Report called for investigation into the scientific basis behind tool mark comparisons.[1] Answering this call, the authors have attempted to prove or disprove the hypothesis that tool marks are unique to a single tool. Recently, it was demonstrated that a statistical algorithm could, in most cases, discern matching and non-matching tool marks made at different angles by sequentially numbered screwdriver tips.[2] Moreover, in the cases where the algorithm misinterpreted a pair of marks, an experienced forensics examiner could discern the correct outcome. While this research serves to confirm the basic assumptions behind tool markanalysis, it also suggests that statistical analysis software could help to reduce the examiners workload.

This led to a new analysis approach that relies on 3D scans of screwdriver tip surfaces at the micrometer scale from an optical microscope. These scans are carefully cleaned to remove noise from the data acquisition process, and then they are assigned a coordinate system that mathematically defines angles and twists in a natural way.

The marking process is then simulated by using a 3D graphics software package to impart rotations to the tip and take the projection of the tips geometry in the direction of tool travel. The edge of this projection, retrieved from the 3D graphics software, becomes the virtual tool mark. Using this method, virtual marks are made at increments of 10 degrees and compared to a scan of the real tool mark. The previously developed statistical package performs the comparison, comparing the similarity of the geometry of both marks to the similarity that would occur due to random chance.[2] Finally, the method informs the forensics examiner of the angle(s) of the best matching virtual mark, allowing the examiner to focus his/her mark analysis on a smaller range of angles and twists.

The preliminary results seem promising. The virtual marking software is capable of importing and cleaning a 3D tool tip in about a minute and producing a virtual mark profile in a matter of seconds. The statistical mark-to-mark comparison can also be performed in seconds. These time estimates are from a standard desktop computer with a 3.20 GHz processor and an inexpensive graphics card. Therefore, the proposed method is capable of making comparisons at several angles in a reasonable amount of time.

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Tool Marks, Impression Evidence, Software Development

A195 The Examination, Evaluation, and Identification of Fired 9mm Cartridge Cases Fired from 1,500+ Different GLOCK® 9mm Semiautomatic Pistols Manufactured Over a 20-Year Period

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After attending this presentation, attendees will understand some principals of utilizing both optical, confocal microscopy and computational pattern recognition in the examination and identification of fired GLOCK® 9mm cartridge cases to include fired cases that were obtained from recently manufactured consecutive firearms slides.

This presentation will impact the forensic science community by demonstrating the ability to identify fired cartridge cases to specific firearms.

Over the last several decades, forensic examiners have struggled with the fact that there is no accepted methodology to generate numerical proof that would independently corroborate morphological conclusions regarding impression evidence such as tool marks. The purpose of this research is to take a step toward developing standardized methodologies that can objectively evaluate tool markcomparison and identification. This presentation will impact the forensic and legal community by presenting the results of research conducted over a 20-year period that involved the identification and inter comparison of over 1,500 fired cartridge cases.

In this research, 617 GLOCK® Model 17 & 19 9mm semiautomatic pistols were obtained from the then Indianapolis Police Department (IPD) and test fired to obtain four fired cases from each pistol. The test fired cases were microscopically examined, evaluated, and intercompared using forensic optical comparison microscopy to determine if the casings could be individualized. Subsequentially, 700 additional cartridge cases—recently manufactured over a five-year period—were added to the research. Using forensic optical comparison microscopy, the combined 1,275 test fired cases were microscopically examined, evaluated, and compared if the fired cases could be individualized. A report of this research experiment was presented at the 2011 annual training seminar of the Association of Firearm and Tool Mark Examiner (AFTE). An additional 225+ fired cases have been added to the existing research cases and examined to determine if they are identifiable to themselves and to the exclusion of the other cases.

Additionally, this research is to take a step toward developing standardized methodologies that can objectively evaluate tool markcomparison and identification. Fifty-eight primer shear marks on 9mm cartridge cases, fired from four GLOCK® Model 19 pistols, and a series of 9mm cases test fired from recently manufactured consecutive GLOCK slides were collected using high-resolution, white light confocal microscopy. The resulting three-dimensional surface topographies were processed with outlier and form removal before a cubic spline filter was used to extract all "waviness surfaces"—the essential "line" information familiar to firearm and tool mark examiners. Taking the mean of all profiles that made up each surface summarized the primer shear waviness topographies. The mean profiles were then subjected to Principal Component Analysis (PCA) for dimensional reduction and Support Vector Machines (SVM) for profile-gun associations. Using 10,000 bootstrap resampling iterations, PCA-SVM required only six "synthetic" features (6D) to produce an estimated identification error rate of 0% on a larger data set of assumed similar statistical properties. At the 95% level of confidence, Conformal Prediction Theory (CPT) coupled with SVM showed an empirical error rate of 7%, slightly higher than the long run guarantee of 5%. With these results, suggestions are made for practical courtroom application of CPT for assigning levels of confidence for SVM identifications of tool marks recorded with confocal microscopy.

Forensic firearms examiners have routinely identified fired bullets and cartridge cases with suspect firearms over the past 100+ years, including numerous studies involving consecutively manufactured components. Conversely, firearms examiners have routinely excluded fired components (bullets and/or cartridge cases) during this same time period. In this research project, examination of the fired cartridge cases against each other, validated that each was identifiable and unique. Additionally, the utilization of confocal microscopy and computational pattern recognition in examining some of these fired cases—including several from consecutive manufactured slides—further validates the ability of a trained examiner to differentiate between various fired components.

This presentation will impact the forensic and legal communities by demonstrating the ability of qualified examiners to evaluate and inter compare a significant number of fired cartridge cases (over 2,250,000 examinations) using conventional optical microscopy. Additionally, the research to take a step towards developing standardized methodologies that can objectively evaluate tool mark comparison and identification with the addition of confocal microscopy will be of great value to the forensic and legal community.

Firearms ID, Manufacturing, Legal Issues

A196 Ballistics Evidence in the JFK Assassination: Just the Facts!

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After attending this presentation, attendees will learn about the documented ballistics evidence in the JFK assassination, and how modern forensic science techniques have impacted the understanding of the case. Attendees can implement the forensic information learned into their research studies and investigation of the assassination.

This presentation will impact the forensic science community by providing attendees with a comprehensive knowledge of the ballistics evidence in the JFK assassination. New information reported by respected investigators from the academic, forensic scientific, legal, and medical communities will impact attendees in terms of their knowledge and competence pertaining to this case.

After nearly fifty years of study, the assassination of President John F. Kennedy remains an unsolved case in the eyes of many forensic experts and the general public. Within months of the 1963 assassination, and, in particular, after the release of the Warren Commission Report in September 1964, doubts began to emerge as to the veracity of some of the conclusions in the Report. The Commission's

findings concluded that Lee Harvey Oswald had acted alone in assassinating the President and assured the public that there had been no conspiracy.[1]

By the mid-1960s, several books and media articles raised legitimate questions regarding the lone assassin theory.[2,3,4] Following a high-profile investigation and trial led by New Orleans District Attorney Jim Garrison (1969) and several Congressional investigations related to the assassination, including the Rockefeller Commission (1974), the Church Committee hearings (1975-76), and the House Select Committee on Assassinations (HSCA, 1977-79), much new forensic evidence was brought to light. The last official verdict, released by the HSCA in 1979, concluded "on the basis of the evidence available, that President John F. Kennedy was probably assassinated as a result of a conspiracy."[5]

With the founding of the Assassination Archives and Research Center (1984) and the Coalition on Political Assassinations (1994), which served as national networks for serious researchers into the JFK (and other) political assassinations, and augmented by materials being obtained under the Freedom of Information Act (FOIA), a growing demand for the release of assassination records led to the creation and subsequent extension of the Assassination Records Review Board (AARB), which held the power to order Federal agencies to supply all materials considered to be Kennedy assassination records to the National Archives and Records Administration (NARA) by a specified date. During its tenure, the AARB oversaw the release of more than four million pages, the largest release of classified documents in U.S. history.[6]

The newly released AARB files provided JFK assassination researchers with a windfall of new forensic information, including pertinent medical, ballistics, photographic, acoustics, and trace evidence. This presentation will report and discuss much of that evidence, including X-ray images of the President's skull, bullet fragments recovered from the limousine, and the state-of-the-art digital replication of the Zapruder film original. A discussion focused on the ballistics evidence, which interconnects with the aforementioned forensic information, will be presented in a logical manner based on reliable factual evidence. The Hon. John Tunheim, Chairman of the Assassination Records Review Board, described the JFK assassination as "a giant jigsaw puzzle with a lot of missing pieces."[7] It is hoped that after hearing this presentation, attendees will be able to put some of those pieces back in the puzzle.

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Ballistics, JFK Assassination, Forensic

A197 Assessing the Degree of Similarity Between Accidental Patterns on Shoeprints Associated With Wearers That Participate in Shared and Independent Activities

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After attending this presentation, attendees will understand the degree of similarity between accidental characteristics based on the context in which footwear is used, how accidental characteristics change with wear over time, and how imaging science and semi-automated numerical methods can assist with comparisons.

This presentation will impact the forensic science community in many ways, from the determination of false positives and false negatives, assessing the divergence of an accidental pattern with continued wear, and gauging the reliability of using imaging science and semi-automated numerical methods to quantify the similarity in accidental patterns that vary with continued use and wearer-context.

The goal of this research is to address the similarity and change in correlation between accidental patterns that develop on the outsoles of shoes worn by individuals that participate in shared versus independent activities. To carry out this research, two groups of volunteers were solicited and provided with new/approved footwear. The first group was asked to wear the footwear while repeatedly participating in shared group activities over a seven-month period of time, while the second group was permitted to wear the approved footwear while carrying out daily independent activities. At predetermined step-intervals participants submitted their footwear for analysis, which consisted of the following data: (1) acquisition; (2) registration; (3) segmentation; (4) processing; and finally, (5) comparison. Steps 1 – 5 resulted in a 731-dimensional feature vector (per scan) that described the accidental pattern associated with each outsole. In total, 1,018 feature vectors were created, generating 34,218 pair-wise comparisons, wherein the similarity between each pattern was defined using the correlation metric. The pairwise comparisons generated six major probability density functions that describe the correlation of Known-Matches (KM), Known Non-Matches (KNM), and known-matches between feature vectors separated by continued wear.

Data analysis consisted of three phases. The first phase compared the density of similarity scores for known-matches across groups. In theory, this was a quality assurance measure since group-differences between known-matches was not anticipated. However, the results indicate that the correlation metric is sensitive to the total number and size of accidentals populating the feature vectors, which can be related to the physical size of the shoe, and the types of activities participated in while wearing the footwear. As such, it is reported that the differences in the correlation of KM comparisons across groups can be explained by the variation in shoe size, and the fact that the shared group performed restricted activities.

The second phase of data analysis compared the density of correlation scores for known-matches that differ with usage, in an effort to determine the degree to which continued wear decreases measured similarity. This question is of interest to the forensic community since exemplars worn for days or weeks after the commission of a crime may show loss and acquisition of accidentals not present on the questioned print deposited at the crime scene. The results of this study indicate that if willing to accept the chance of 1 false positive out of every 100 comparisons, then 0 miles of additional wear will lead to 99 out of 100 true positives. However, if the questioned and exemplar known-matches differ by just 10 miles of wear, the additional wear decreases the chance of obtaining a true positive to 23 in 50 for the shared group, and 43 in 50 for the independent group.

The third phase of data analysis compared the density of correlation scores for KNMs across groups. The goal was to determine if participation in a shared activity increases the similarity of accidental patterns on the outsoles of shoes worn by different individuals (and at

the extreme, a rise in the number of false positive associations). The results indicate that it is likely to encounter a negative correlation 58% of the time when comparing KNMs between individuals that participate in random activities, and 64% of the time when comparing KNMs between individuals that participate in shared activities. However, when a positive correlation is computed, it is more likely to be greater in magnitude for KNMs in the shared group.

Footwear, Shared Activity, Accidentals

A198 A 3D Imaging Device for Tire and Footwear Impressions

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After attending this presentation, attendees will see a description of a new prototype device to capture three-dimensional (3D) height images as well as two-dimensional (2D) color images of tire and footwear impressions.

This presentation will impact the forensic science community by introducing a new device and a new method for non-destructively capturing the details of impression evidence by producing high-resolution 3D depth images with metric measurements co-registered with high-resolution color images.

The anticipated manner of using this device will be to take the device to the field and scan impression evidence. The result of this is an HD video on an SD card that is then processed in the crime lab in order to generate the 3D impression image and the 2D color image of the evidence. The resulting 3D impression evidence can be used to match suspect footwear or tires. Through the use of automated matching algorithms that utilize the 3D impression image, a goodness-of-fit measure can also be calculated.

The design of the device is based on a class of methods known as "shape from structured light." It uses a camera/line laser assembly that can estimate the surface depth values along a single laser line in the image based on the deformations of the laser line caused by the surface shape. This results in a one-dimensional (1D) depth image in one frame of the video along the laser line. The depth image of the entire impression is generated by moving this camera/line laser assembly along a motorized rail at a precisely controlled and constant speed and estimating the depth values along successive laser lines. The desired 3D image of the impression is obtained by stacking these one-dimensional depth values along the axis aligned with the rail movement direction.

The camera used in the camera/laser assembly is a high definition (HD) video camera. Scanning of the surface consists of recording the video at 30 frames per second (fps) as the camera/laser assembly moves along the rail. This video is later processed in the crime lab to compute the impression depth image. The HD video camera is oriented such that the highest resolution of the camera image is perpendicular to the rail motion, thus increasing the resolution in this perpendicular direction. The image formation model along this direction is perspective projection. The resolution of the image along the rail motion depends on the speed with which the camera assembly is moved—the slower the motion, the higher the resolution of the depth image in this direction. Moreover, the image formation model in the direction of the rail movement is orthographic projection. This allows long span impression evidence such as tire track impressions to be scanned in a single attempt without the need to consider camera models and the necessity to stitch together multiple 2D images.

In order to obtain the proper metric scale of the impression evidence, a calibration object is placed next to the impression and this object is scanned as part of the data. This eliminates the need for pre-calibration of the system. Instead, the calibration object in the captured video and the object's true dimensions are used in order to compute the impression surface shape with true scale information.

The resulting device is portable, easy to use, is non-destructive of the evidence, saves time at crime scenes compared to current methods, and provides metric information about the surface shape. The device is designed to be able to digitize long tire track impressions (up to 1.75m) in a single scan without the need to capture multiple images and stitch them together. The 3D images produced by the device can have a resolution in depth of 1mm to 0.5mm, capturing fine details. The image resolution along the rail motion can be as little as 0.0438mm. The image resolution perpendicular to the rail motion at a distance of 500mm is about 0.2369mm. The basics of the design, how the device works, and some impression digitization results using it will be presented.

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Impression Evidence, 3D Image, Depth Image

A199 Quantitative Algorithm for Digital Comparison of Torn Duct Tape

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After attending this presentation, attendees will understand some of the principles of objective, quantitative methods. These mathematical methods are currently in use by the academic engineering community, and can be applied to the area of physical matching in regards to duct tape examinations. The attendees will have greater appreciation for some of the complex tasks that can be conducted with modern digital imaging software.

This presentation will impact the forensic science community by establishing the first objective numerical methodology that can be successfully applied to physical matching of duct tape, its results meeting one of the key tenants of the NAS Report "*Strengthening Forensic Science in the United States: A Path Forward*."

The National Academy of Sciences (NAS) cited the need to establish the scientific basis demonstrating the validity of forensic science methods. In support of this, we have developed a quantitative algorithm for the digital comparison of torn and cut duct tape ends to evaluate their end match. This algorithm is programmed to perform a series of software routines that will result with each torn duct tape end profile displayed in a graphical format and then the profile of the torn duct tape end is compared to all the other duct tape specimens using residual analysis. The key idea of this research is that the true match will have a statistically significant lower sum of the squared residuals than all the other test specimens that do not match; the associated non-matching specimens will have much larger residual values. Having numerical results will enable us to derive meaningful statistical information that can describe significant differences between the matching and non-matching duct tape ends. This research involves the inter-comparison of samples from a set of 100 pairs of torn duct tape specimens. Inter-comparison of this set of 100 pairs resulted in 10,000 inter-comparisons. These torn duct tape specimens are also unique in that each of these specimens has been used in a previous statistical research study to look at end matches by four experienced Graduate Student Researchers (GSRs); these GSRs were required to inter-compare duct tape specimens as part of a training protocol. The GSRs had to identify those that matched and those that did not match within the set of 100 pairs of duct tape, which all four GSRs were able to do correctly. Using the quantitative mathematical algorithm, it is expected to provide numerical data correlating matching and non-matching specimens which can be related to the findings of the analyst. The end product of this research will be a quantitative and statistically rigorous guideline for end match comparison.

In this analysis, the left side of a torn tape specimen was called the "Exemplar" tape and it was compared to the right side of a torn tape specimen which was called the "Sample" tape. The procedure for

processing each tape specimen consists of a series of steps beginning with scanning the specimens into the computer at 1,200 DPI. Next, using MATLAB®, the North and South boundaries of each tape are selected, followed by defining the torn area of interest. The algorithm then performs a series of image manipulation and provides a graphical profile of the torn ends. Due to minute thread fragments extending from the tape that can interfere with residual analysis, the algorithm performs a series of extraction and dilation routines on the torn profile. The Exemplar profiles are then compared to the 100 Sample profiles in the database using residual analysis. The hypothesis is that the pair of tapes with the lowest residual value (in pixel^2 x 10^6) should be a matching pair in the set of 100 pairs of tapes. Research supported this hypothesis, in that a pair with the lowest residual values for the set was determined to be the matching pair. There are still many issues that could enhance future research in this area such as the "entropy of the torn specimen." The current algorithm treats each tear or cut in the same manner, but in reality a tape with a complex tear pattern is much more significant. This type of tape, because of its randomness, should be given a more unique residual value than a tape with a very simple tear pattern, even though the residual value for the two tapes could be similar. Although this development work used the engineering software MATLAB®, the end product will be an executable program that does not necessarily require the use of MATLAB®.

Duct Tape, Physical Matching, Image Analysis

A200 Taiwan Banknote Drug Contamination

Chun-Yu Chen, MS, and Frederick P. Smith, PhD, Univ of New Haven, Forensic Science Program, 300 Boston Post Rd, West Haven, CT 06516*

After attending this presentation, attendees will understand some potential forensic implications of banknote drug contamination focusing on specific results from the analyses of New Taiwan Dollar (NTD) banknotes, comparing these data with drug-use demographics and drug seizures in Taiwan, as well as published reports concerning currency from the U.S. and other countries.

This presentation will impact the forensic science community by furthering the understanding of external contamination issues, which may be germane to medico-legal investigators who evaluate the significance of drug test results in: (1) questioned deaths; (2) public health concerns/forensic epidemiology; (3) drug crimes; and, (4) drug-use toxicological tests where reliability is based on assumptions about external contamination, particularly when forensic investigators rely on examinations of surfaces in the workplace and elsewhere to interpret the significance of the presence of drugs on these surfaces.

New Taiwan dollars (n = 200, $100 denomination) were collected from five major metropolitan areas representing a geographical distribution within the Republic of China (Taipei, Taipei County, Tauyuan, Taichung City, and Kaohsiung), taking precautions to insure the integrity of collection procedures. From each general location, multiple bills were retrieved from actual transactions in commercial locations including outdoor markets near temples, food stands, betel nut outdoor stands, convenience stores, hotels (including in red-light district), fuel stations, private karaoke and movie booth rentals, entertainment, and gaming locations (slot machines).

Initial presumptive testing of banknotes was conducted using disposable Drugwipe II immunoassay devices (formerly Securetec, now Affiniton LLC) for the following classes of controlled substances/metabolites: cannabis, amphetamines, cocaine, and opiates. Further extraction followed for instrumental confirmation. Negative and positive controls were included as additional tests to exclude false-positive results from laboratory contamination. Deuterated internal standards (d_3-cocaine, d_5-amphetamine, d_5-methamphetamine, d_5-methylenedioxymethamphetamine, d_4-ketamine, d_3-morphine, and d_3-Δ^9-tetrahydrocannabinol) were added to the banknotes, and they were air dried. Analytes were removed from specimens using 10mL 0.1 N hydrochloric acid, and then separated using solid phase (SPE) columns, derivatized using pentafluoropropionic anhydride, and analyzed by ion trap CI-GC/MS.

When extracted and screened with Drugwipe II, a significant number of bills showed positive results for cannabis (69.6%), amphetamines (65.2%), opiates (26.1%), and cocaine (8.7%). Since Taiwan is an island-nation located on the edge of Southeast Asia and Northeast Asia, with a well-developed shipping system (both maritime and air transport) and extensive movement of goods within these regions and worldwide, there is ample opportunity for drug trafficking. The relationship between illicit drug seizures and drug-use demographics within Taiwan provide insight into NTD banknote analyses reported in this research. These data show quantities of controlled substances (ng/banknote) which exceed the limits of detection and limits of quantification for analyses of other external surface matrices such as hair (expressed in ng/mg) and sweat (expressed in ng/mL extract), as well as inanimate objects, for which there is no mandated standard practice or cutoff for reporting a surface as "positive" for a drug. These results suggest caution when ascribing drug use conclusions based on surface testing when contamination cannot be excluded.

Drugs, Contamination, Currency

A201 Statistical Comparison of Mass Spectral Data in the Identification of Amphetamine-Type Stimulants

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After attending this presentation, attendees will be familiar with a statistical procedure that has been developed for the comparison of mass spectral data.

This presentation will impact the forensic science community by providing a method to assign statistical confidence to the comparison of mass spectra. Specifically, application of the statistical procedure in the comparison of mass spectral data generated for standards and case samples containing amphetamine-type stimulants will be presented.

Gas chromatography-mass spectrometry is commonly used for the analysis of controlled substance samples. While this technique provides both retention time and mass spectral data for the comparison of the submitted sample to a suitable reference standard, the identification of amphetamine-type stimulants can be challenging. Firstly, since many of these compounds have the same phenethylamine base structure, the resulting mass spectra are very similar, especially in the lower mass region. Additionally, these compounds readily fragment under typical electron ionization conditions and, oftentimes, the molecular ion is not present in the spectrum, making identification based on molecular mass difficult. These challenges can be overcome by derivatizing the sample or by using alternative ionization procedures; however, both of these options increase the time necessary for sample analysis, which is not desirable in a forensic laboratory. As a result, amphetamine-type stimulants are typically analyzed in the underivatized form and under electron ionization conditions.

The resulting mass spectrum of the controlled substance in the submitted sample is then compared visually to the spectrum of a known reference standard, either analyzed by the laboratory or obtained from a mass spectral database. However, since the publication of the National Academy of Sciences Report in 2009, there has been a trend toward a more statistical evaluation of forensic evidence, which is not commonplace in controlled substance analysis and identification. The aim of this research was to develop a suitable procedure that provides such a statistical basis for the comparison of mass spectral data, thereby addressing the current limitations.

The developed procedure uses Student's t-tests and classical probability theory to compare two mass spectral fragmentation patterns. Briefly, the Student's t-test is used to compare the two spectra at every mass-to-charge ratio (m/z) in the scan range. The calculated t-statistic (t_{calc}) is then compared to the critical t-value (t_{crit}) at the required confidence level. If, at any m/z in the range, t_{calc} is greater than t_{crit}, then

the two spectra are considered to be statistically distinguishable. If t_{calc} is less than t_{crit} at all m/z, the two spectra are considered to be statistically indistinguishable. In these cases, classical probability theory is used to determine the random match probability, which is the probability that the spectral pattern occurs by chance.

In this presentation, the procedure is applied for the comparison of mass spectra of amphetamine-type stimulants. Spectra of case samples containing amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, and phentermine were obtained from an accredited forensic laboratory and compared to spectra of appropriate reference standards. Each case sample spectrum was statistically associated to the corresponding reference standard at the 99.9% confidence level. Random match probabilities were also calculated and, in each case, the probability of the spectral pattern occurring by chance was infinitesimally small (e.g., 1.7×10^{-39} for the comparison of a case sample containing amphetamine to an amphetamine reference standard).

Mass spectra of the case samples were also compared to all other reference standards, using the same procedure. In general, statistical discrimination of the samples from unrelated standards was observed at the 99.9% confidence level, which is the most rigorous confidence level for discrimination. However, a case sample containing methamphetamine was discriminated from the phentermine reference standard at the slightly lower confidence level of 99.0%. These two substances have the same molecular mass and, since they are structural isomers, distinction of the two based on mass spectral data is challenging. Despite the similar fragmentation patterns, methamphetamine and phentermine were distinguished with high confidence.

While demonstrated for the comparison of controlled substances data, the statistical procedure developed can be readily applied to other types of forensic evidence in which the comparison of mass spectral data is necessary.

Mass Spectral Data, Statistics, Amphetamines

A202 Pharmaceutical Identifier Confirmation Via AccuTOF™ DART®

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The goal of the presentation is to show how pharmaceutical identity can be confirmed using tablet physical identifiers and mass spectrum from an Accurate Mass Time-Of Flight Mass Spectrometer, coupled with the Direct Analysis In Real Time ion source (AccuTOF™ DART®).

This presentation will impact the forensic science community by using physical identifiers and the AccuTOF™ DART® to confirm pharmaceutical identity that will eliminate the use of GC/MS and effectively reduce analysis time. This will prove helpful in laboratories with large backlogs and will simplify the confirmation process.

Pharmaceutical tablets are analyzed in a forensic-controlled substances laboratory on a daily basis and comprise a large amount of the casework. The current method for identifying pharmaceuticals at the Virginia Department of Forensic Science (VaDFS) begins with physical identification, using sources such as Ident-A-Drug, The Drug Identification Bible, and RxID software. Thin Layer Chromatography (TLC) is then utilized to screen for the presumptive identity of the pharmaceutical. Alternatively, the AccuTOF™ DART® is utilized for pharmaceutical screening at DFS and is the most definitive screening test. Finally, the tablet is analyzed using a Gas Chromatograph and Mass Spectrometer (GC/MS) to confirm the identity.

The AccuTOF™ DART® is composed of an ambient ion source coupled with an accurate mass time-of flight mass analyzer. The ambient ion source allows samples to be analyzed at atmospheric pressure requiring minimal sample preparation without extraction. The sample molecule is protonated via the DART® and enters the TOF mass analyzer. The mass of the protonated molecule is accurate to ± 5.0 mDa with a much higher resolving power than the nominal mass produced in a quadrupole MS. Higher ion source voltages cause fragmentation through collision-induced dissociation (CID), producing a spectrum similar to an electron ionization source.

This project seeks to investigate the confirmation of pharmaceuticals using the AccuTOF™ DART® after preliminary identification through tablet and capsule markings. The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) advises confirming the identity of pharmaceutical tablets through a Category B technique, such as physical identification, and a Category A technique, such as mass spectrometry. Differentiating between pharmaceuticals with the same molecular mass can be difficult when using the AccuTOF™ DART® because the same protonated molecule is produced. For example, codeine and hydrocodone have a molecular mass of 299 Da and will produce a protonated molecule with a mass of 300.160 Da on the AccuTOF™ DART®. By increasing the source voltage, unique ion fragments are expected to appear in the spectrum and will aid in distinguishing between drugs with identical protonated molecules. Cocaine and scopolamine have a molecular mass of 303 Da; when fragmented, cocaine will produce an ion with a mass of 182 Da and scopolamine will produce an ion with a mass of 138 Da. These unique fragments, along with others, differentiate cocaine and scopolamine. Reproducibility of spectra from one data file to another were experimentally determined and statistically evaluated to ensure accurate identification. For pharmaceuticals that do not have an obvious difference in ion fragments, Principal Components Analysis (PCA) was used for differentiation. PCA is a statistical analysis used to discriminate between similar data values. Linear Discriminant Analysis (LDA) was performed on the PCA plot to determine differences between data values. LDA models categorical data through linear combinations to determine differences. It provides an objective, measurable value to the PCA plot.

Results were obtained using DART® parameters of 275°C gas stream temperature, and Orifice one voltages of 30V and 90V. The 90V spectra provided different fragment ions and abundance values for pharmaceuticals with the same molecular mass, thus making it possible to differentiate between hydrocodone and codeine and, similarly, morphine and hydromorphone. A separate analysis was performed on the isomers of codeine: heterocodeine, hydrocodone, neopine, and pseudocodeine. All five were differentiated using PCA and LDA. Over 500 pharmaceutical tablets and capsules, collected from VaDFS casework, were analyzed and their pharmaceutical identifiers were accurately confirmed in this experiment. If the AccuTOF™ DART® spectrum identifies a different drug than what was indicated by the pharmaceutical identifiers, then the sample would be confirmed using the original analysis scheme with the use of GC/MS.

Using physical identifiers and the AccuTOF™ DART® to confirm pharmaceutical identity will eliminate the use of GC/MS and effectively reduce analysis time. This will prove helpful in laboratories with large backlogs and will simplify the confirmation process.

Pharmaceutical, DART®, Mass Spectrometry

A203 HPLC-UV Determination of Synthetic Cannabinoids in Herbal Products

Laura A. Ciolino, PhD, FDA, Forensic Chemistry Center, 6751 Steger Dr, Cincinnati, OH 45237*

After attending this presentation, attendees will learn about a validated HPLC-UV method for the determination of synthetic cannabinoids in various sample matrices including bulk plant materials and powders will be fully described. Suitability of the HPLC-UV method has been demonstrated for compounds representing several structural classes of synthetic cannabinoids, including the naphthoylindoles (JWH-018, JWH-073, JWH-200, AM-2201, JWH-122), cyclohexylphenols (CP 47,497 and CP 47, 497-C8 homolog), benzoylindoles (RCS-4), phenylacetylindoles (JWH-250), and others (HU210, UR144, XLR11). Quantitative results for a series of herbal product forensic samples will be presented.

This presentation will impact the forensic science community by providing the forensic chemist with an analytical method for determinating synthetic cannabinoids in herbal smoking products.

A validated HPLC-UV method for the determination of synthetic cannabinoids in various sample matrices including bulk plant materials and powders will be fully described. Suitability of the HPLC-UV method has been demonstrated for compounds representing several structural classes of synthetic cannabinoids, including the naphthoylindoles (JWH-018, JWH-073, JWH-200, AM-2201, JWH-122), cyclohexylphenols (CP 47,497 and CP 47, 497-C_8 homolog), benzoylindoles (RCS-4), phenylacetylindoles (JWH-250), and others (HU210, UR144, XLR11). Quantitative results for a series of herbal product forensic samples will be presented.

In 1990, it was discovered that a receptor found in brain cells mediates the CNS effects of Δ^9-tetrahydrocannabinol including mood and cognition, and this cannabinoid receptor was designated CB1.[1,2] In 1993, a second cannabinod receptor (CB2) was identified, with CB2 receptors being associated with immune cells and their modulation.[2,3] Over the last 20 years, hundreds of cannabinoids have been synthesized by various pharmaceutical or university scientists as part of drug development efforts or pharmacological studies involving CB1 and CB2 receptors. From a chemical standpoint, the synthetic cannabinoids comprise several structural classes including naphthoylindoles, cyclohexylphenols, benzoylindoles, phenylacetylindoles, and others.

Herbal smoking products with added synthetic cannabinoids have emerged as common substances of abuse within the last decade. The EMCDDA reported that sales of these products commenced in Europe during the years spanning 2004 – 2006, while CBP reported the first U.S. occurrence in 2008 as a formal import entry.[4,5] Reports from DEA and the UNODC have shown marked increases in the sales and abuse of cannabinoid-laced herbal products in the years which followed.[6,7] The products have been sold via the internet or specialty stores under a variety of names, among which "Spice" and "K2" are two well-known examples. The products are frequently labeled to contain "aromatic potpourri" or "incense," and consist of dried plant leaves loosely packaged in foil packs. Damiana leaf, marshmallow leaf, and mullein leaf are among the plant materials commonly used. Investigation into the product sources indicates that the cannabinoids are deposited onto plant surfaces via spraying, soaking, and/or mixing with cannabinoid solutions, after which the solvent(s) are evaporated. In July 2012, the FDA Safety and Innovation Act was signed into law, making several classes of cannabimimetic agents (with CB1 receptor activity) Schedule 1 substances in the US.[8]

In this work, an HPLC-UV method for determination of synthetic cannabinoids in bulk plant materials and powders was developed and validated. The method uses acetonitrile for extraction/dissolution, followed by separation on a commercial phenylhexyl stationary phase. UV detection provides excellent sensitivity with limits of detection (LODs) less than 10mg/g for many synthetic cannabinoids which possess highly conjugated chromophores. Spike/recovery studies demonstrate good to excellent recovery (75 -110%) for synthetic cannabinoids from damiana leaf, marshmallow leaf, and mullein leaf over a wide range of cannabinoid content (0.1 – 75mg/g). The method has been applied to a series of case-related herbal products with determined amounts ranging from <0.1mg/g to >100mg/g.

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Synthetic Cannabinoids, Herbal Smoking Products, HPLC-UV

A204 Investigation Into Cyclohexanone as a Schiff-Base Derivatizing Agent for the Detection of Cathinones With Gas Chromatography/Mass Spectrometry

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After attending this presentation, attendees will have a better understanding of the use of cyclohexanone as a derivatizing reagent for cathinone compounds.

This presentation will impact the forensic science community by providing a simple method for screening and identification of the target cathinone analogues in illicit samples using cyclohexanone as a Schiff-base derivatizing agent and Gas Chromatography-Mass Spectrometry (GC/MS).

*"*Bath salts*"* have recently become an illicit drug problem across the United States. Originating in the United Kingdom, *"*bath salts*"* have spread nationwide and are currently being classified as illegal substances of abuse by most states. Illicit drug suppliers are trying to circumvent the control of these substances by altering the structural arrangement of the compounds creating new analogues. As a result, forensic scientists are scrambling to develop methods to detect and identify these illicit substances. Consequently, cyclohexanone was investigated as a derivatizing reagent for the detection by GC/MS of thirteen structurally similar *β*-keto-phenethylamine compounds which have evidence of abuse. Cathinone, cathine (norpseudoephedrine), methcathinone (ephedrone), 4'-methylmethcathinone (mephedrone), 4'-fluoromethcathinone (flephedrone), dimethylcathinone (diethylpropion), ephedrine, pseudoephedrine, norephedrine (phenylpropanolamine), methylpseudoephedrine, N-ethylcathinone (ethcathinone), 4'-methoxymethcathinone (methedrone), and 3,4-methylenedioxymethcathinone (methylone) were all examined using GC/MS. Derivatization of the cathinones by cyclohexanone via Schiff-base formation was simple and proved to be an efficient method. Using this method, it is possible to achieve separation and identify many structurally similar cathinone-related compounds in mixtures.

A GC/MS method for screening or confirming 13 cathinone-related compounds using cyclohexanone as Schiff-base derivatizing reagent was developed. The method is capable of separating the 13 cathinone compounds tested with baseline resolution in less than 11 minutes. Primary and secondary amines will react with cyclohexanone via a Schiff-base reaction to form two distinct different types of derivatives. Tertiary amines, such as diethylpropion and methylpseudoephedrine, do not react with cyclohexanone to form a derivative and also do not interfere with the method. Cyclohexanone forms an imine derivative with primary amines and enamine derivatives with secondary amines. Cathinone forms both an imine and enamine when it is derivatized using cyclohexanone. Due to the presence of the *β*-hydroxyl functional group, ephedrine and pseudoephedrine (both being secondary amines) react with cyclohexanone to form oxazolidines. The reactions are all reproducible and show good chromatographic behavior on the gas chromatographic column stationary phase tested.

GC/MS data was acquired using a quadrupole Mass-Selective Detector (MSD). The gas chromatographic oven temperature

parameters were set with an initial temperature of 120 C which then increased 15 C/min to 250 C for a total run time of 10.67 minutes. The column used was a 30m x 0.25mm x 0.25µm phenylmethylsilicone capillary column (Rxi® -5Sil MS) using Helium as a carrier gas with a linear gas velocity of 38cm/sec. Methanol was used as the solvent and a sample volume of 1µL was injected in the split mode with a split ratio of 50:1. A retention time optimization study provided the most advantageous separation conditions.

Using cyclohexanone to form the Schiff-base derivative, a more complicated mass spectrum can be created and used to identify and differentiate similar cathinone compounds. The increased fragmentation also aids in providing more structural information about the compound. This method can be used to screen unknown samples and identify multiple cathinones in one sample.

Forensic Science, Forensic Chemistry, Cathinones

A205 A Retrospective Critical Evaluation of Statistical Techniques Utilized in Clandestine Drug Profiling

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After attending this presentation, attendees will recognize the need to consider which statistical techniques are appropriate prior to analysis being carried out and gain awareness of issues relating to some of the statistical methods previously used for drug profiling.

This presentation will impact the forensic science community by encouraging consideration of statistical approaches to data processing prior to analysis, identifying the appropriateness of statistical techniques currently utilized in drug profiling, and questions that need to be answered relating to some of these.

Selection of statistical methods for clandestine drug profiling is often not considered in detail prior to application and where consideration is made, the basis is frequently identification of common practice or testing a variety of approaches against known results. There needs to be reference to the underlying assumptions or distributional requirements when deciding upon the appropriate statistical technique.

Reliable, robust methods for identification of illicit compounds are essential to many involved in law enforcement. This process includes not only direct identification of the compounds but is also coupled with the determination of methods of manufacture and ability to associate seizures. Physical and chemical profiling of illicit drugs aims to establish whether or not there are links between seizures.[1,2,3]

A wide variety of statistical methods have been applied to both the physical and analytical measurements of illicit drug samples in attempts to establish similarity, or lack thereof, between samples.[1,5,6] Approaches to selection of the technique to utilize vary from “what everyone else does” to “what gives the best fit to what we know to be true.”

In fact, many statistical techniques are based upon assumptions relating to the data set being analyzed or rely upon properties of the data set to produce results. Yet these techniques are utilized without consideration of this in drug profiling.

The extent of evaluation of both analytical and statistical approaches varies by drug type — synthetic, semi-synthetic, or natural — and by drug. For synthetic drugs, there has been extensive research into drug profiling, including a harmonized analytical and statistical method for amphetamine profiling.[1] Research relating to semi-synthetic drugs (cocaine and heroin) is less abundant and, for natural drugs such as cannabis, is limited, as identified by Groger *et al.*[4]

This abstract presents a review of the statistical techniques which have been utilized in profiling of clandestine drugs from each of the synthetic, semi-synthetic, and natural drug groupings. The assumptions for each of the approaches followed are identified and evaluated in the context of current understanding of the properties of the drug profiles. Such consideration allows the identification of key issues in selection and evaluation of appropriate statistical methodologies across a wide range of illicit drugs.

For the first time, this research will critically evaluate the use of statistical methods within the context of drug comparison. Foreseeable impact of this is recognition of the need to consider which statistical techniques are appropriate prior to analysis being carried out. This will allow inferences to be correctly drawn by forensic scientists and law enforcement bodies when profiling clandestine drug seizures allowing appropriate and reliable both by the laboratories but also between laboratories and across borders.

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Statistics, Drug, Profiling

A206 Development of a Surface-Enhanced Raman Spectroscopy Method for the Detection of Benzodiazepines in Urine

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The goal of this presentation is to show the development of surface-enhanced Raman spectroscopy method for the analysis and detection of trace quantities of benzodiazepines in urine. The optimization of various parameters of this technique as well as the limits of this method will also be discussed.

This presentation will impact the forensic science community by providing information regarding Surface-enhanced Raman spectroscopy (SERS) method and how it has shown the applicability of SERS for the detection of trace quantities of benzodiazepines extracted from toxicological samples and the use of the technique over a wide range of compounds. SERS is more specific than currently used immunoassays as it provides spectral information for the compounds present. Also, this technique has higher sensitivity and permits detection of drugs such as lorazepam, which have poor cross reactivity when using standard immunoassays.

Benzodiazepines are medications for anti-anxiety and anti-depression that are commonly prescribed. These drugs are prominent in the commission of drug-facilitated sexual assaults due to their effects on the central nervous system such as drowsiness, amnesia, confusion, and impaired coordination. Due to their potency, a low dose of these compounds is often administered to victims; therefore, the target detection limit for these compounds in biological samples is 50ng/mL, which is well below therapeutic concentrations.

Surface-enhanced Raman spectroscopy (SERS) has previously been shown to detect trace quantities of compounds, such as nicotine, in aqueous solutions. This technique has the advantage of overcoming

the low sensitivity and quenching the unwanted fluorescence effects seen with conventional Raman spectroscopy. SERS spectra are obtained by applying a compound of interest onto a SERS-active metal substrate such as colloidal metal particles or metal films. In this case, the colloidal particles are spherical gold nanoparticles in aqueous solution. SERS signals can be further increased with the addition of aggregate solutions. These agents are salt solutions which cause the nanoparticles to amass and form hot-spots which increase the signal intensity. Chlorine salts generally provide the greatest enhancement for two reasons. The chlorine ions displace the stabilizing agent to cause aggregation and they affect the ionic strength of the surrounding solution, changing the surface charge of the substrate, therefore increasing the signal intensity.

Spiked urine samples were prepared by adding diluted benzodiazepine and metabolite samples (prepared in 10% methanol) to drug-free urine at a range of benzodiazepine concentrations (1ng/mL – 500ng/mL). A solid phase extraction method specific for benzodiazepines was used. Extraction efficiency was determined by quantitation using direct infusion mass spectrometry. Aqueous colloidal dispersions of gold spherical nanoparticles were prepared using a modified Lee Meisel 1% sodium citrate reduction method. Particle size and shape were confirmed with an average size of approximately 30nm. Previous work has shown that for benzodiazepines, an aggregate solution made of $MgCl_2$ prepared at a concentration of 1.67 M provided the highest signal intensity at the lowest drug concentration and was used in this study. Aggregate solutions were added to colloidal dispersions followed by the addition of extracted benzodiazepine samples and SERS spectra were obtained.

Overall this method allows for the detection of a wide variety of benzodiazepines and their metabolites. The presence of individualizing spectral peaks provides a high degree of specificity for sample determination. The technique is sensitive with a limit of detection of 2.5ng/mL and linear over several orders of magnitude for the drugs chosen.

This method has shown the applicability of SERS for the detection of trace quantities of benzodiazepines extracted from toxicological samples and the use of the technique over a wide range of compounds. SERS is more specific than currently used immunoassays as it provides spectral information for the compounds present. Also, this technique has higher sensitivity and permits detection of drugs such as lorazepam, which have poor cross reactivity when using standard immunoassays.

Benzodiazepin, SERS, Drug Analysis

A207 Extracting and Characterizing Cannabinoids From Cellusose Storage Cards – A Convenient Sampling Method for Marijuana Samples

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After attending this presentation, attendees will be aware that marijuana vegetable matter can be simply sampled by rubbing on cellulose collection cards, compact and stable for extended periods. Chemical constituents can be recovered from these cards and characterized using GC/MS.

This presentation will impact the forensic science community by affecting the way large marijuana submissions are stored, with cellulose storage cards providing reference samples that can yield useful comparative intellegence data even years after the initial sampling.

Cellulose-based cards are known to serve as compact and stable storage media for many types of biological fluids and plant samples. The utility of such storage has been demonstrated for *Cannabis sativa* by successful recovery and genotyping of DNA from plant material rubbed on such cards.[1] The sampling is done by rubbing the leafy material directly on the card and later recovery can be done by punching small plugs out of the stained cellulose sampling area. The study demonstrates that such plugs could also be used to look at the chemical constituents (cannabinoids) such as tetrahydrocannibinol (THC) that can be recovered from this type of storage. Recovery of THC would indicate that such samples came from material that had some drug potency and qualitative examination of other recoverable cannabis plant components might aid in comparing and sourcing of the sampled plant material.

The study initially concentrated on developing an efficient recovery scheme for cannabinoids from the storage cards. Several difference solvents including petroleum ether, petroleum ether/ethyl acetate, acetonitrile, dimethylformamide (DMF), and tetrahydrofuran were examined.[2] Cards supplied by the National Marijuana Initiative (NMI) of the High Intensity Drug Trafficking Authority were used.[3] These were cards that had been examined for DNA by Dr. Coyle and her students here at the University of New Haven.[1] An extraction scheme was developed that successfully recovered small amounts of cannabinoids from the cards.

Nineteen cards received from National Marijuana Initiative (NMI) that had been used to sample drug grade cannabis vegetable matter were first examined.[3] Peaks in the gas chromatograms at the retention times of THC and cannabinol were observed. These were also examined using Gas Chromatography/Mass Spectrometry (GC/MS) and the peaks were confirmed to be THC and cannabinol. The chromatographic programs on the two instruments were adjusted so that retention times were almost identical on each instrument. Cards sampled at Nebraska Wesleyan using local wild cannabis, assumed to be hemp type, were also tested.[4] These cards showed a significant peak, in most cases, at the retention time of cannabidiol. This peak was too weak to confirm in all cases but did show, in the majority, a mass spectrum consistent with cannabidiol.

It was found that Gas Chromatography (GC) of the card extracts provided chromatograms with better signal to noise ratio than the Total Ion (TI) chromatograms obtained on GC/MS. However, mass spectra that allowed identification of the primary cannabinoid peaks were obtained.

A Single-Ion Monitoring scheme that shows promise to extend the sensitivity of our GC/MS instrument for such samples was developed. This should allow identification of other naturally occurring cannabinoids, the presence of which should allow comparison of plant samples preserved on cards to gain some useful intelligence information. Because many of the samples were at or near detection limits, the researchers are also looking for ways to improve recovery or chromatographic behavior.

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4. Received from Dr. Heather Coyle Miller courtesy of Dr. Jerry Bricker, Biology Department, Nebraska Wesleyan University, Lincoln, Nebraska.

Marijuana, Sampling, GC/MS

A208 2013 Update From the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)

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After attending this presentation, attendees will be knowledgeable of SWGDRUG activities during 2012, including revisions to SWGDRUG recommendations, new supplemental documents, and other work products in development.

This presentation will impact the forensic science community by providing current recommendations, new developments, and future

projects and direction of SWGDRUG as it relates to the analysis of seized drugs.

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) was formed in 1997 in a joint effort between the U.S. Drug Enforcement Administration (DEA) Office of Forensic Sciences and the Office of National Drug Control Policy (ONDCP). The mission of SWGDRUG is to recommend minimum standards for the forensic examination of seized drugs and to seek their international acceptance. This presentation will provide attendees with an update on SWGDRUG activities during the year 2012, including revisions to SWGDRUG recommendations, new Supplemental Documents, and other work products in development.

In January 2012, version 6 of the SWGDRUG Recommendations was current and available to the general public via the group's website (www.swgdrug.org). Currently, the SWGDRUG core committee is working on revising recommendations pertaining to the use of reference materials. Laboratories throughout the world are encountering difficulties obtaining and verifying new reference materials. These tasks have become more overwhelming with the appearance of new designer drugs like synthetic cannabinoids and bath salts. Revisions to the recommendations will include minimum procedures for the verification of these materials, as well as alternative guidance for when newly emerging materials are encountered and verification is limited to structural elucidation procedures.

In 2012, revised versions of the SWGDRUG mass spectral library were made available to the general community. Currently, the library contains more than 1,600 spectra, including many of the recently encountered synthetic cannabinoids, bath salt compounds, and their isomers. Laboratory analysts throughout the world can download this library from the SWGDRUG website and onto their laboratory instruments. Feedback from analysts and library users continues to be highly positive. The library will continue to be updated on a regular basis and contributions from the forensic community are strongly encouraged.

During 2012, the SWGDRUG core committee finalized Supplemental Document SD-4. This document provides three examples of measurement uncertainty calculations for purity determinations. A draft of the document was made available for public comments via the SWGDRUG website during July – September. Comments received from the public will be reviewed and addressed during the next core committee meeting (January 2013). This presentation will include a general summary and discussion of this supplemental document.

Also during 2012, the SWGDRUG core committee finalized, approved, and posted Supplemental Document SD-5. This document contains two examples of laboratory reports showing how minimum SWGDRUG recommendations can be fulfilled. The two report examples included in SD-5 will also be discussed during this presentation.

Presently, members of the SWGDRUG core committee are also working on the following projects:

- Online resource center for drug analysis training
- Assessing the current controlled substance analogue issue

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, Drug Analysis

A209 DART®-MS Collision Induced Dissociation (CID) for Structural Analysis of Synthetic Cannabinoids

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After attending this presentation, attendees will understand ambient ionization mass spectrometry, basics on synthetic cannabinoid detection, and how to use fragments for structural identification.

This presentation will impact the forensic science commmunity by demonstrating a cutting-edge technique applied to a current problem, and how chemical analysis can be performed without any sample preparation, extraction, or derivatization that are often required for GC/MS and LC/MS analyses.

The emergence of numerous cannabinoid designer drugs has been tied to large spikes in emergency room visits and overdoses. Identifying these substances is difficult due to: (1) the fact that the compounds are novel, structurally related, and do not usually test positive in drug screens; (2) the rapidity with which they appear on the market; (3) the absence of standard protocols for their identification; and, (4) the customized and extensive sample preparation/extraction and analysis procedures required to demonstrate their presence. Direct Analysis in Real Time Mass Spectrometry (DART®-MS) is a technique that utilizes an atmospheric pressure ion source that produces a heated stream of metastable helium species directed at sample surfaces to vaporize and ionize liquids or desorb and ionize molecules from solid surfaces in open air under ambient conditions. Semi-volatile substances, like the cannabinoids mixed on the plant material, desorb from the leaf surface and are ionized. The metastable helium atoms initiate a cascade of ion-molecule reactions in ambient air to provide protonated water clusters as a chemical ionization reagent to produce parent ions, $[M+H]^+$, from the cannabinoid drugs.

DART®-MS provided high mass accuracy, to 0.0001 Da, establishing the presence of the cannabinoids JWH-122, JWH-203, JWH-210, RCS-4, and AM-2201, alone and as mixtures of at least two cannabinoids. Mass spectra were acquired by simply suspending a small portion of sample between the ion source and the mass spectrometer. The ability to test minute amounts of sample is a major advantage when limited amounts of evidentiary material are available. This method circumvents time-consuming sample extraction, derivatization, chromatographic, and other sample preparative steps required for analysis by more traditional methods. The high throughput capabilities of DART®-MS enable the active components of designer drugs to be detected more quickly, reducing the time necessary to triage analytical evidence. Therefore, exploitation of this method has the potential to contribute to more timely criminal prosecution. In addition, DART®-MS employing CID conditions provided confirmatory structural information that was useful in characterizing the various isobaric cannabinoid analogs.

Specifically, DART®-MS CID induces fragmentation of the protonated parent ions when the electrode voltage at the inlet orifice to the mass spectrometer is increased, demonstrating the utility of fragmentation patterns for distinguishing among closely related structures. The researchers used in-source CID to produce product ions corresponding to the synthetic cannabinoid molecules desorbed from dried leaves. CID analysis illustrated that closely related compounds are likely to fragment in a similar fashion, but their inherent structural differences will result in unique fragments that vary enough between the singular cannabinoids that can serve as a means to better identify each substance. Closely related compounds fragmented with both consensus peaks and unique fragments, such that both their structural similarities and differences provided multiple diagnostic peaks that permitted additional confidence toward identification of each substance. DART®-MS spectra were acquired to rapidly differentiate among

synthetic cannabinoids contained within "herbal" products purchased locally in New York (U.S.). The spectra exhibited the following parent ion peaks and product ion fragments unique to each cannabinoid that corresponded to major structural features: JWH-122 (*m/z* 356.1997, 298, 214, 169, 141), JWH-203 (*m/z* 340.1466, 342, 214, 127, 125), JWH-210 (*m/z* 370.2179, 312, 214, 183, 155), RCS-4 (*m/z* 322.1778, 164, 214, 186, 135), and AM-2201 (*m/z* 360.1757, 284, 232, 155, 127).

DART®-MS, Cannabinoids, Ambient Ionization

A210 Profiling Methylamphetamine Synthesized Using Precursors Extracted From Proprietary Cold Medication Through "Hypo and Moscow" Synthetic Routes Using Isotope Ratio Mass Spectroscopy (IRMS)

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After attending this presentation, attendees will understand basic principles of isotope ratio mass spectrometry, and how it is used in the isotope profiling of methylamphetamine produced via two similar synthetic processes. Isotope ratio mass spectrometry provides an "isotope fingerprint" of a chemical molecule which may be useful in discriminating between precursor source for the methylamphetamine synthesized and this is explored in detail.

This presentation will impact the forensic science community by providing useful insight in the use statistical approach and isotopic analysis to discriminate between batches of methylamphetamine synthesized from pure grade materials and batches of methylamphetamine synthesized from precursors extracted from cold medication using different solvents and other pharmaceutical-grade essential chemicals, i.e., iodine tinctures, hydrogen peroxide. Two routes of synthesis were investigated in this research: (1) Moscow route; and, (2) Hypophosphorous route. These routes were chosen due to their popularity in clandestine synthesis of methylamphetamine. Other key catalyst such as red phosphorous was extracted from matchbooks and iodine crystals from iodine tinctures using hydrogen peroxide. Most of the methodology of the extraction of the precursor and extracting the essential chemicals were obtained from clandestine literature. This is to produce methylamphetamine as similar to the "street samples" as possible. The methamphetamine synthesized from the two routes was analysed by the Isotope Ratio Mass Spectrometry (IRMS).

This work exposes the variation in light stable isotopic values (C,H,N) derived from the analysis of methylamphetamine synthesized from two popular clandestine routes, the Hypophosphorous and Moscow routes. The final products were repetitively synthesised using precursors, catalysts, and reducing agents that were derived from household products and cold medications. *Pseudo*ephedrine was extracted using three different solvent systems, from Sudafed®, an over-the-counter cold medication widely available in the United Kingdom. Methylamphetamine was also prepared from laboratory grade *pseudo*ephedrine. Six repetitive batches of the final products were produced in each case to provide within and between batch variations and providing a total of 48 samples (24 for each route)

The isotope ratio mass spectrometry provides an "isotope fingerprint" of a chemical molecule which is useful in discriminating between batches, determining geographic origin, and manufacturing routes. The IRMS has received a considerable amount of attention in the forensic community over the last decade, which is also used extensively as an analytical tool in the natural and life sciences field. Isotope ratio mass spectrometry analysis (IRMS) is potentially useful in the comparison and discrimination of batches of methylamphetamine produced from the same precursor materials and different synthetic routes. There appears to be a significant effect encountered as a result of the precursor extracting solvent and, to our knowledge, this is the first time IRMS has been applied to articulate these differences. IRMS was used to specifically address the potential to link the methylamphetamine product to either of the synthetic routes (Moscow or Hypo) or to link the final product by precursor extraction solvent. The ability of IRMS to discriminate the inter and intra batch variation of methylamphetamine synthesized from both clandestine routes will also be discussed.

Methylamphetamine, Illicit Synthesis, IRMS

A211 Headspace-Gas Chromatographic-Mass Spectrometric (HS-GC/MS) Analysis of South American Commercial Solvents and Their Possible Use in the Illicit Conversion of Cocaine Base to Cocaine Hydrochloride

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After attending this presentation, attendees will understand the methodology utilized in the clandestine processing of cocaine hydrochloride, the solvent analysis of illicit cocaine hydrochloride samples, and the conclusions made concerning types and quantities of solvents used in processing of cocaine hydrochloride.

This presentation will impact the forensic science community by providing a method by which the amount of occluded solvents in the crystal matrix of cocaine hydrochloride may be used to establish the actual ratios or makeup of solvents utilized in illicit productions, and also indicate the diversion of commercial solvents in seized illicit cocaine hydrochloride samples.

Illicit cocaine hydrochloride is normally prepared by the addition of a water miscible solvent containing hydrochloric acid to a water immiscible solvent containing dissolved cocaine base. Cocaine hydrochloride quickly crystallizes from the mixture and is recovered via filtration. In general, this process is utilized by South American clandestine cocaine laboratories; however, there are unique variations in the solvents utilized.

The two types of organic solvents typically used for the production of cocaine hydrochloride are water immiscible solvents, referred to as "Solvent-A", and water miscible solvents, referred to as "Solvent-B." Both Solvent A and Solvent B can be either pure or a mixture of compatible solvents. The various solvents in the final (combined Solvents A and B) mixture are trapped within the matrix during cocaine hydrochloride crystal growth and are referred to as "occluded" solvents. The rapid precipitation of cocaine hydrochloride is favorable to the occlusion of organic solvents within the crystal matrix. In previous studies, a wide variety of occluded solvents have been observed in illicit cocaine hydrochloride samples. However, due to differential inclusion, the ratios of occluded solvents in cocaine hydrochloride do not necessarily represent the actual ratios of the solvent combinations that were used.

Over the past 30 years, the United States has imposed strict controls on chemicals in order to prevent or reduce the diversion of solvents to illicit drug production. Several South American countries have implemented similar controls on common chemicals utilized in the production of illicit drugs such as cocaine. However, some of these restricted solvents are still diverted for illegal practices. To date, there have been no studies to establish possible correlations between occluded solvents observed from illicit cocaine hydrochloride samples and commercially manufactured organic solvents in South America. The presented research attempts to determine if diversion of commercially manufactured organic solvents in South America for the clandestine production of cocaine hydrochloride is occurring and can be documented.

Thirty-five samples of commercial solvents were obtained from four chemical manufacturing companies in South America. Each sample was individually analyzed for its component makeup and quantitated

using static headspace-gas chromatography-mass spectrometry. After obtaining a chemical profile for each solvent, individual batches of cocaine hydrochloride were prepared from cocaine base using solvents or solvent mixtures that are frequently employed in clandestine laboratories. Solvents and/or solvent mixtures selected or prepared were similar to several of the commercial products. The cocaine hydrochloride produced in-house was analyzed to determine the correlations of occluded solvents before and after processing. Quantitative data corresponding to commercial solvents and the resulting cocaine hydrochloride will be presented.

Forensic Science, Cocaine, Occluded Solvents

A212 Identification of Cannabimimetrics and Cathinones by GC/EI-MS and LC/ESI/QTOF-MS

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After attending this presentation, attendees will learn how Liquid Chromatography Electrospray Ionization Quadrupole Time-Of-Flight Mass Spectrometry (LC/ESI/QTOF-MS) and Gas Chromatography Electron Impact Ionization Mass Spectrometry (GC/EI-MS) can be applied to the analysis of cannabimimetrics and cathinones that are currently found in "legal high" products.

This presentation will impact the forensic science community by emphasizing an analytical approach for identifying synthetic designer drugs using accurate mass determination along with EI fragmentation elucidation. The findings and methodology will provide forensic laboratories the ability to identify alternative means for detecting and identifying designer drug substances.

The use of illegal synthetic cannabinoids and cathinone derivatives has become popular in recent years. Many of these products are often marketed using labels such as "bath salts" and "herbal blends" and are packaged with a disclaimer that they are not for human consumption. Labels on the packaging do not often reflect the actual contents, misleading the consumers that the products are safe and legitimate. Consequently, these substances are increasingly popular as legal alternatives to illicit psychoactive substances for recreational drug users. The demand is further enhanced by their widespread and easy availability on the Internet and head shops across America.

The majority of cases encountered by forensic chemists are analyzed utilizing traditional methodologies such as GC/MS, GC/FID, FTIR-ATR, and LC/MS. Identification of unknowns has been largely dependent on mass spectral libraries from SWGDRUG, Cayman Chemical, and scientific discussion forums such as Forendex. Moreover, due to the vast variety of synthetic drugs, authenticated reference standards may not be easily available, thereby increasing analysis time and cost of unusual cases. The introduction for newer and efficient methodologies into the classical workflow is essential. The objective of this study is to demonstrate how synthetic substances (target or unknown) can be identified using accurate mass spectrometry analysis alongside with EI fragmentation elucidation.

Preliminary results of this study demonstrated that ESI/QTOF-MS software-based accurate mass determination provided highly specific mass-to-charge spectral data with accuracies spanning in the milli-Dalton range for parent and fragment ions. The method employs chromatographic separation using Zorbax Extend C18, 2.1mm x 50mm, 1.8um particle size stationary phase. TOF-MS full-scan spectra were acquired in positive mode over 50-1000 m/z scan range using reference masses m/z 121.0509 and 922.0098. Additional TOF-MS parameters were set as follows: fragmentor voltage at 150 V; capillary voltage at 4000 V; skimmer voltage at 65 V; nebulizer pressure at 50 psi; gas temperature at 350 C; and gas flow rate at 13 L/min. Collision-induced-dissociation (CID) of precursor ions were obtained in targeted MS/MS mode using a collision cell with nitrogen as a collision gas.

Applications presented will include the analysis of some of the emerging cannabimimetrics such as AM-1220, AM-2233, UR-144, and XLR-11. In addition, cathinone derivatives such as butylone, ethylone, pyrrolidinopropiophenones, and 4-methylbuphedrone will be discussed. The results showed that ESI/QTOF-MS can be used in complement with GC/MS to provide principal means of identification of designer cathinones and synthetic additives in herbal mixtures. By applying these techniques, forensic laboratories will have the ability to characterize designer synthetic drugs.

Cannabinoids, Cathinones, QTOF/MS

A213 Rapid Screening of Synthetic Cannabinoids With NMR and DART®-MS

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After attending this presentation, attendees will understand the fundamental principles of Direct Analysis At Real-Time Mass Spectrometry (DART®-MS) and Nuclear Magnetic Resonance (NMR) spectroscopy, specifically their applications for rapid detection of synthetic cannabinoids in herbal incenses. Attendees also will appreciate the power of combining two spectroscopic methods to promptly elucidate accurate structures of cannabinoids with no ambiguity.

This presentation will impact the forensic science community by introducing a combined spectroscopic approach to quickly solve the challenges in identifying synthetic cannabinoids and isomers found in herbal incenses or potpourri, also known as "spice" or "fake pot."

The recently-passed S.3187 included five classes of synthetic cannabinoids into Schedule I controlled substance list. It creates great challenges for forensic scientists to rapidly screen these "cannabimimetic agents" in numerous forms of herbal incenses due to the similarity of isomer or analog structures. Newer compounds are being synthesized promptly to circumvent the ban, which exacerbates the analytical difficulties for forensic labs, some of which are already experiencing backlogs.

Our hypothesis is to combine the rapid speed of DART®-MS with H1 NMR to rapidly screen and concretely confirm the identities of synthetic cannabinoids in herbal products with minimum sample preparation. The mass spectra provide molecular weights as well as fragment information. NMR spectra confirm the structure with no ambiguity. The whole process takes less than two hours. Novel isomers and analogs can be quickly identified and structures confirmed, sometimes without the presence of standards.

As suggested in previous literature, DART® is an atmospheric ionization technique that can be used to instantly ionize illicit drugs sprayed herbal base materials with no sample preparation. The limitation, however, is the difficulty of DART-MS to elucidate structural isomers. With the help of NMR, the isomeric or analog structures can be confirmed.

A commercially available high resolution DART®-TOF mass spectrometer system was used for the direct analysis of all samples. The DART® was operated in positive ion mode using helium gas at a gas heater temperature of 300° C. The powder samples were introduced into the DART® gas stream as a coating on the outside of a glass rod. For the herbal samples, three random pieces of plant material were selected from a given sample bag and then held in the DART® gas stream with forceps. The CAD experiments were done by varying the mass spectrometer cone voltage at 0.2sec intervals to induce fragmentation. For the NMR study, a simple methanol (3mL) extraction was employed to remove synthetic cannabinoids from 50mg of solid herbs, before paper filtration, solvent evaporation and redissolving in NMR solvent such as $CDCl_3$ or d-6 acetone. After one-hour H1 NMR scan, the chemical shift values from synthetic components were detected and used to confirm DART®-MS finding. The chemical shift values of signature peaks were analyzed to identify synthetic cannabinoids. Our study suggests that most signature peaks from naphthoyl and benzoyl indoles are in 7-8.5ppm or 4-4.2ppm ranges, which do not interfere with the herbal background signals from blank

herbal extracts. A flow chart was developed to quickly identify the cannabinoids based on their signature chemical shift values.

With the combination of DART®-MS and NMR, 30 herbal samples and seven powder samples from distributors were quickly screened for synthetic cannabinoids. Three of the powder samples were found to be mislabeled and the other three contaminated with other cannabinoid(s). Synthetic cannabinoids were found in all but one herbal incense. Among the herbal samples investigated, AM-2201, JWH-122, JWH-210, and RCS-4 were found to be the predominant ingredients.

In conclusion, accurate identification of synthetic cannabinoids in powder and herbal samples can be rapidly achieved with DART®-MS along with NMR confirmation. The simple NMR method can differentiate isomers such as JWH-019, JWH-180, and JWH-122. These combined spectroscopic methods can help forensic chemists to achieve accurate identification within two hours which increases the analytical throughput and helps to decrease sample backlog.

Cannabinoids, NMR, DART-MS

A214 Visualizing Depleted Latent Fingerprints Using Columnar Thin Films

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After attending this presentation, attendees will have gained knowledge of the use of the Conformal-Evaporated-Film-By-Rotation (CEFR) technique to deposit Columnar Thin Films (CTFs) on depleted latent fingerprints for development and visualization. This study aims to determine the sensitivity of the CEFR development technique by developing fingerprints which have been successively placed in order to reduce the amount of sebaceous material left behind (i.e., as a depletion series). Comparison to traditional methods is made by using depletion series of split fingerprints on several different forensically relevant surfaces. The selected forensically relevant substrates are those which previous research has shown that traditional development techniques work as well or better than the CEFR development technique.

This presentation will impact the forensic science community by adding another option for latent fingerprint development in situations where the fingerprint quality is low and traditional development techniques are not adequate.

Determining the sensitivity of this fairly new development technique may impact the forensic science community by adding another option for latent fingerprint development in situations where the fingerprint quality is low and traditional development techniques are not adequate.

By the CEFR technique, a CTF is deposited onto a latent fingerprint on a substrate that is affixed to a rotating platform in a vacuum chamber. In the vacuum chamber, a source material is evaporated by resistive heating to produce a collimated vapor flux. The vapor condenses on the rapidly rotating substrate, thereby creating a thin film that entombs the fingerprint. This thin film comprises columns with diameters on the nanometer length scale. The CTF produces an observable contrast between the fingerprint ridge detail and the underlying substrate, and thus allows the fingerprint to be visualized by its surface topology rather than by mechanical or chemical interactions.

A study was carried out to determine the sensitivity of the CEFR technique by using depletion series. For each series, sebaceous secretions were collected on a pre-cleaned finger and then fingerprints were deposited without renewal of the sebaceous material on the finger. The second, fourth, sixth, eighth, tenth, and twelfth fingerprints were each placed on a substrate of the same kind. Each substrate was prepared for split fingerprints, with one half of the fingerprint being developed by the CEFR technique and the other half by a traditional technique. The substrates used were black nylon, white nylon, black ABS plastic, white ABS plastic, brass, and stainless steel. Based on previous research, the evaporant materials utilized were chalcogenide glass and tris(8-hydroquinolinato)aluminum (Alq3). Traditional techniques utilized were: red fluorescent powder, black powder, black magnetic powder, cyanoacrylate fuming, and cyanoblue with cyanoacrylate fuming.

The depletion series were obtained by wrapping the fingertip in a clear thin plastic wrap to eliminate the interference of eccrine secretions being produced from the pores in the friction ridge skin. In a preliminary study, the eccrine secretions caused some fingerprints deposited later in the depletion series to have more material than earlier depleted fingerprints. This caused the depletion series to be ineffective and inconsistent between different trials. The inconsistency was eliminated when the plastic wrap was used.

Results obtained were qualitatively and quantitatively, examined. Qualitatively, photographs of the developed fingerprints were visually graded, and quantitatively the photographs were examined using the Universal Latent Workstation (ULW). The fingerprints examined with the ULW were graded using a custom algorithm which calculates the amount of definitive minutiae in each fingerprint as determined by the quality map produced with the ULW's extended feature set. Depletion series on brass, developed with a CTF, were qualitatively and quantitatively superior to those developed by traditional techniques, but depletion series on hard plastics gave mixed results.

Further research is still being conducted with depletion series on other substrates and with other evaporant materials. The CEFR technique will also be compared with vacuum metal deposition using the split print method in the future. The CEFR technique is also currently being evaluated for the development of partial bloody fingerprints and fingerprints on fired cartridge casings. Other fluorescent evaporant materials are also being compared to Alq3.

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Depleted Fingerprint, Conformal-Evaporated, Columnar Thin Film

A215 MALDI/TOF-MS for Chemical Analysis of Fingerprint Residues Using Conventional Fingerprint Development Methods

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After attending this presentation, attendees will understand the benefit of measuring chemical information from fingerprint residue, and how image chemical information is obtained from latent fingerprint residue using conventional latent fingerprint development techniques coupled with Matrix-Assisted Laser Desorption Ionization/Time-Of-Flight Mass Spectrometry (MALDI/TOF-MS).

This presentation will impact the forensic science community by demonstrating the ability to extract measurable identifiable chemical markers detected in fingerprint residue with the potential to assist in forensic investigation.

Chemical information gained from analysis of latent fingerprints, "touch chemistry," at a crime scene might provide investigative or other forensically relevant information. Studies reported in the literature demonstrate the ability to gain intelligence information (smoker/nonsmoker, drug exposure, and ingested drugs of abuse) from chemicals recovered within fingerprint residue using MALDI/TOF-MS. One of the main challenges with fingerprint imaging is the ability to integrate MALDI/TOF-MS with current protocols used by latent fingerprint examiners. For this presentation, two experimental approaches are described for imaging latent fingerprint residue. For the first design, conventional methods used by latent fingerprint examiners will be evaluated as a way of visualizing a print and acting as the matrix for MALDI in order to ascertain chemical information that can be measured in fingerprint residue. For the second experimental design, a new method incorporating a MALDI matrix will be used to compare with conventional approaches.

For the experimental design, fingerprint residues were developed with powder, cyanoacrylate fumed or lifted. Control fingerprints included grooming the fingers by rubbing across the nose or neck to include sebaceous secretions. A second groomed print was deposited next to

the control print after handling powder from either an over-the-counter pharmaceutical or explosive. The prints were developed with black powder, black magnetic powder, black magnetic nanoparticle powder, black nanomagnetic powder, or cyanoacrylate fuming. The lift experiments followed the same procedure for depositing and developing latent prints on metal surfaces. An alternative method included using a MALDI matrix sprayer to deposit alpha-Cyano-4-Hydroxycinnaminic Acid (CHCA) on dusted, cyanoacrylate fumed, and lifted fingerprints.

Fingerprint images from direct deposition and lifts gave distinct differences between the control fingerprint and the fingerprint after handling the drug or explosive. Powders of trinitrotoluene (TNT) along with three pharmaceutical products (containing aspirin, acetaminophen, and ibuprofen) were used for the handling experiments and both positive and negative ionization mode was used to collect fingerprint images. The pharmaceuticals all formed $[M+Na]^+$ and $[M+K]^+$ adducts in positive ionization mode. TNT formed an $[M]^-$ for negative ionization mode. From the MALDI image of the handled powders, the detail of the fingerprint ridges were not observed but the distribution of the powders across the finger was shown. In order to view the ridge details of the fingerprint, target ions from the groomed fingerprints were extracted. For all fingerprints analyzed, some ridge detail was observed after development by either powder or cyanoacrylate prior to MALDI analysis. Fingerprints developed with powders used less laser power and provided less chemical interferences than cyanoacrylate fuming. The cyanoacrylate method worked well for detecting the handled compounds, but the remaining spectral chemical information from the fingerprint was from the cyanoacrylate polymer. Initial experiments using nanoparticle powder visually demonstrated greater detail of the fingerprint compared to conventional black magnetic powder. However, all powders demonstrated the ability to work as MALDI matrix in the handled experiments.

With the addition of the MALDI matrix, compounds of interest were more efficiently ionized at a lower laser power and enhanced signal to noise response for the handled pharmaceuticals and explosive. By incorporating CHCA into the fingerprint method, protonated or deprotonated ions were observed for acetaminophen, aspirin, ibuprofen, and TNT. Less sodium and potassium adducts were also observed, potentially allowing for structural MS/MS information to be obtained. The conventional and new methods both demonstrated the ability to obtain chemical information from fingerprint residues using MALDI/TOF-MS.

MALDI/TOF-MS, Fingerprint Residue, MALDI/TOF-MS Imaging

A216 Evaluation of Universal Latent Workstation for Automated Minutiae Detection

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After attending this presentation, attendees will understand that the minutiae in a single person's fingerprint will change from one deposit to another on similar and different substrates. They will also learn that computer software programs used to automatically extract minutiae in a fingerprint are not always accurate and their assigning of minutiae changes from impression to impression as well as by the positioning of the area of interest selection box.

This presentation will impact the forensic science community by demonstrating the ability of the universal latent workstation to automatically extract minutiae in two consecutively deposited fingerprint exemplars and two consecutively deposited latent fingerprints.

A fingerprint is made up of ridge paths that form minutiae points which a latent print examiner uses in comparisons and identifications. Some examiners use software programs in the evaluation phase before comparisons to find minutiae and judge latent print quality that can aid in reducing searching time. There are a number of vendors that sell latent print search software that can also extract latent print minutiae. However, these vendors all use different computer algorithms to assign search print minutiae which cannot be readily compared because of proprietary source codes. Unfortunately, these vendors sell to multiple agencies at the local, state, and national level. This presents a problem of scanning a fingerprint using one vendor's software and trying to find a potential match in another system. The Universal Latent Workstation (ULW) was developed by the Advanced Technology Unit, Criminal Justice Information Services (CJIS) Division of the FBI to aid in solving this problem. ULW is a program developed to identify and extract minutiae in fingerprints so the print can be encoded once and searched many times in a number of vendor systems. ULW uses an algorithm called MINDTCT which locates and records minutiae.

The purpose of this preliminary study is to determine what changes, if any, are detected by ULW when minutiae are auto extracted in two sequentially recorded inked prints and in two sequentially lifted controlled latent prints using black fingerprint powder. It is expected that there will be relatively high accuracy in auto extraction in both the sequential prints and that there will be a low number of incorrectly located and labeled minutiae with few artifacts; to wit: identified and plotted minutiae where none is present.

Several volunteers supplied record inked fingerprints, controlled latent prints, and actual latent fingerprints in the collection of a ground truth fingerprint sample set for another study. Only the right index finger, finger number two, was recorded 30 times on three standard fingerprint cards using black ink. The volunteers also provided latent prints on glass slides with both uncontrolled and controlled pressure. Another latent print was collected by adding one pound of lead sheeting to a beaker and having the volunteer momentarily lift the beaker from and return it to a countertop. Two sequential recorded inked prints were randomly chosen for this preliminary study as was a single controlled latent from a glass slide. The latent print was visualized using black fingerprint powder and lifted twice. The second lift was accomplished without the reapplication of powder. The inked and latent prints were cropped and scanned at 1000ppi.

It is expected that when latent fingerprint examiners comparing fingerprints from the same individual, even if they are on different substrates or if the print has been lifted multiple times, the same minutiae should be detected. However, for a software program like ULW, it will be expected that when those same fingerprints are encoded, there will be variability in the number of minutiae assigned depending on the quality, clarity, and contrast of the print as well as the number of times the print was lifted and where the area of interest selection box is positioned.

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.

Ground Truth; Fingerprints; Universal Latent Workstation

A217 Understanding the Concept of "Sufficiency" in Friction Ridge Examination

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After attending this presentation, attendees will have a better appreciation of the type of information that latent print examiners use during their examinations; more specifically, how examiners use that information to form opinions on the source of latent prints, while also realizing the amount of variability that exists between multiple examiners observing the same set of prints.

This presentation will impact the forensic science community by providing data on the variance in the feature selection, and the way these features are used by examiners when forming opinions on the same set of prints.

The examination of friction ridge skin impressions relies on the observation and comparison of various friction ridge features, such as the general pattern of the ridge flow, minutiae, number and location of

ridge pores, and shape of ridge edges. The observation and comparison of such friction ridge features between skin impressions recovered on crime scenes and control prints from individuals enables latent print examiners to form opinion on the identity of their source.

Following the method formalized by David Ashbaugh, examiners generally claim to rely on: (1) the quality and quantity of friction ridge features in agreement; and, (2) the lack of significant differences between two skin impressions (usually a trace and a control impression) to form their opinion.[1] Pairs of impressions displaying *sufficient* features in agreement are deemed to be from the same source; pairs of impressions displaying *insufficient* information in agreement, but no significant discordances, are deemed to be inconclusive; finally, pairs of impressions displaying disaccording features are deemed to be from different sources.

Scientific and legal scholars regularly question the accuracy and reliability of this opinion-based process. In addition, the decision-making process lacks transparency in the sense that examiners cannot describe how the information observed on pairs of impressions is used to form their opinion. Overall, this has led some scholars to raise the possibility for examiners to be subjected to bias.

Recent research projects have aimed at measuring the accuracy of latent print examination and have reported error rates.[2,3] Other projects have resulted in models measuring the quality and quantity of information present in friction ridge skin.[4] Some of these models allow for the objective quantification of the weight of fingerprint evidence in casework. Data gathered during these projects have also been used to support the development of the new SWGFAST Standards for Examining Friction Ridge Impressions and Resulting Conclusions.[5] Nevertheless, the way examiners combine the quality and quantity of information to form their conclusions has only scarcely been investigated.

This particular project aimed at using the tools and results obtained from previous projects to investigate the use of friction ridge features during the very last stages of the decision-making process by latent print examiners. During this project, an online fingerprint comparison training system was used to gather data on the observations made on pairs of fingerprints and the subsequent decisions formed by examiners on the identity of their source. More specifically, approximately 100 examiners were tasked to: (1) compare a series of pairs of latent/control prints; (2) annotate the relevant friction ridge features (quality and quantity); (3) provide their personal evaluation of the quality and quantity of information; and, (4) report their final conclusions.

Several metrics were designed to measure examiners' consistency between the quality and quantity of their annotated features and the decisions made at various stages of the fingerprint examination process on the different test prints. This presentation will report the results of this study; more specifically, we will show the amount of variability within and between examiners when annotating the same set of prints and when forming conclusions on the identity of their source.

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Latent Print, Sufficiency, Variability

A218 Evidence for Expertise in Fingerprint Identification and the Ramifications for the Future Study of Forensic Expertise

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After attending this presentation, attendees will receive an overview of recent criticisms of forensic identification, be updated on the findings of and rationale behind recent studies of expertise in fingerprint identification, and understand the implications of these experiments for expert testimony and public policy.

This presentation will impact the forensic science community by providing a rationale for the design of recent experiments on forensic expertise and put the experimental results in context for the benefit of forensic researchers, examiners, managers, and policy makers.

Although fingerprint experts have presented evidence in criminal courts for more than a century, there have been few scientific investigations of the *human* capacity to discriminate these patterns. In 2009, the National Academy of Sciences (NAS) delivered a landmark report highlighting the absence of solid scientific methods and practices in the forensic science domain.[1] Harry T. Edwards (a senior U.S. judge and co-chair of the NAS Committee) noted that forensic science disciplines, including fingerprints, are typically not grounded in scientific methodology and forensic experts are not bound by solid practices that ensure that the forensic evidence that is offered in court is valid and reliable.[2] The NAS recommended that the U.S. Congress fund basic research to help the forensic community strengthen their field, develop valid and reliable measures of performance, and establish evidence-based standards for analyzing and reporting testimony.

Recently, researchers have investigated the effect of contextual bias on fingerprint examiners; some of the special abilities and vulnerabilities of fingerprint examiners; the effect of technology; statistical models of fingerprint identification; and the accuracy of fingerprint examiners' decisions. Despite this contribution, there are still few peer-reviewed studies directly examining the extent to which experts can correctly match fingerprints to one another, how competent and proficient fingerprint experts are, how and on what basis examiners make their decisions, and what factors affect matching accuracy. The "Identifying Fingerprint Expertise" experiment was designed to find out whether fingerprint experts were any more accurate at matching prints than the average person, and to get an idea of how often they make errors of the sort that could lead to a failure to identify a criminal compared to how often they make errors of the sort that could lead to inaccurate evidence being presented in court.[3] Results show that qualified, court-practicing fingerprint experts are exceedingly accurate (and more conservative) compared with novices, but they do make errors.

In this presentation, a rationale for the design of the experiment will be provided, as well as context for interpreting the results for the benefit of researchers, forensic examiners, forensic managers, lawyers, and judges. It will be argued that fidelity, generalizability, and control must be balanced in order to answer important research questions; that the proficiency and competence of fingerprint examiners is best determined when experiments include highly similar print pairs, in a signal detection paradigm, where the ground truth is known; that determining error rates with system-wide black box studies may be inefficient at best and unnecessary at worst; and that inferring from this experiment the statement, "the error rate of fingerprint identification is 0.68%" would be disingenuous.[4] In closing, the ramifications of these findings for the future study of forensic expertise, and the implications for expert testimony and public policy will be presented.

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Fingerprints, Decision-Making, Expertise

A219 Results of the NIJ Motor Vehicle Theft DNA Demonstration Program Evaluation

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After attending this presentation, attendees will learn the impact of DNA evidence on criminal justice outcomes for motor vehicle theft investigations in two demonstration sites (Dallas and New York), while learning about the experimental design—a randomized controlled trial (RCT)—of forensic laboratory practices applied to actual criminal events.

This presentation will impact the forensic science community by presenting the results of methodologically-rigorous but relatively rare RCT evaluation that measured the impact DNA evidence had on the investigation and prosecution of motor vehicle theft, a common and costly property crime. The information presented will be of interest to laboratory, law enforcement, and court personnel from jurisdictions that are considering expanding the use of DNA evidence beyond serious person crimes and to all who are interested in how forensic science practices may impact criminal justice system outcomes.

DNA testing has been used to aid investigations and prosecutions of serious person crimes since the 1980s. Some jurisdictions have, either routinely or intermittently, extended this practice to property crimes and lesser offenses. In 2009, The National Institute of Justice (NIJ) commissioned a demonstration project to expand DNA evidence collection and testing to motor vehicle theft investigations in two cities: Dallas, TX, and New York, NY. Additionally, the research was funded to conduct an evaluation of this dual-site demonstration program. Using cost-effectiveness analysis, impact analysis, and outcome analysis, this evaluation tested the hypothesis that using DNA evidence to aid motor vehicle theft investigations is more efficient than traditional investigative practices.

Unlike items stolen during domestic or commercial burglaries, nearly 60% of stolen motor vehicles are actually recovered, thereby increasing the chances of obtaining the DNA of recent vehicle operators from surfaces or items in the recovered vehicle. Both of the demonstration sites identified approximately 500 motor vehicle cases where DNA evidence was collected. Researchers assigned those cases to treatment and control cohorts in equal numbers. Physical evidence from cases in the treatment group underwent DNA testing, while evidence in control cases was not tested until the business-as-usual investigation was concluded.

At the case-level, the study tested whether adding DNA to traditional investigative procedures results in more: suspects identified, cases closed with an arrest, cases accepted for prosecution and convictions. In addition, analyses were conducted at the sample-level to evaluate whether there are attributes of treatment group samples that are associated with better (or worse) case outcomes, conditional on DNA testing. This study is only the second RCT ever conducted that measures the impact of forensic evidence on criminal justice outcomes.

The first, completed in 2008, found that when DNA evidence was used to aid property crime investigations, law enforcement was twice as likely to make an arrest when compared to cases where the DNA evidence was not tested. Since motor vehicle theft was excluded from that work, this experiment was a natural next step in testing the cost effectiveness of using DNA to aid criminal investigations of property crimes.

DNA, RCT, Vehicle Theft

A220 Touch DNA on Shell Casing and Cartridges: A Case Study

Nasir A. Butt, PhD, OCME, 11001 Cedar Ave, Cleveland, OH 44106*

After attending this presentation, attendees will learn the importance of DNA analysis on shell casings and live cartridges found on crime scenes, these discoveries ultimately helping to identify the shooter.

This presentation will impact the forensic science community by encouraging the use of touch DNA from shell casings as a link between the crime and the perpetrator since touch DNA can withstand the firing heat released during the discharge of a gun and, with the present PCR technology, can be helpful in solving the crimes.

This presentation will reflect the importance of DNA analysis on shell casings and live cartridges found at crime scenes. Touch DNA found on a shell casing and live cartridge at a robbery of a gas station that resulted in a homicide and the touch DNA on a live cartridge and a gun on a robbery of a dollar store helped identify the shooter.

This case study reflects that the epithelial cells or touch DNA can withstand the firing heat released during the discharge of a gun and, with the present PCR technology, can be helpful in solving the crimes. Firearms are commonly used in a majority of violent crimes. Shell casings at the crime scene are sometimes the only evidence available for the investigation. These shell casings and/or live cartridges are often sent for the analysis of latent prints with almost no success. The chances of losing any touch DNA present on this type of evidence increases when the recovery and collection of touch DNA is delayed. Various studies have shown that the heat released during the discharge of a weapon has very limited effect on the DNA.

In December 2007, an armed robbery of a gas station resulted in the murder of the owner of the business. The police recovered from the scene a 9mm live cartridge and a 9mm shell casing. The only bullet entered the victim's chest and exited his back. The shell casing and the live cartridge were brought to the laboratory for the analysis. The shell casing and live cartridge were swabbed for the analysis of touch DNA. DNA was extracted on the whole swab from each using Organic extraction methods. The extracted DNA was quantified using Quantifiler® human quantification kit using an RT-PCR instrument. Furthermore, the DNA was amplified using an ABI Identifiler® kit. The amplified DNA was loaded onto an genetic analyzer for the detection of the DNA fragments. Fragment analysis was performed using ID software. The STR DNA profiles were developed from the shell casing and the live cartridge.

In January 2008, case detectives submitted two guns and live cartridges from another robbery which took place at a dollar store. Two suspects were developed in this robbery and their buccal swab samples were submitted for analysis. The DNA found on the firearm and the cartridge was consistent with the DNA found on the shell casing and the live cartridge recovered from the December 2007 robbery and homicide scene. The STR DNA profile was consistent with one of the suspects who was later found guilty of both crimes.

The firearm examination and test firing revealed that the cartridge casing recovered from the robbery of gas station was fired from the 9mm gun submitted from the robbery of the dollar store.

The analysis of touch DNA on the shell casings and live cartridges recovered at crime scenes can thus provide a link between the crime, suspect and/ or victim, which might sometimes be the only evidence in a case.

Touch DNA, Shell Casing, STR DNA Analysis

A221 Inter-Agency Collaboration Combating Child Sexual Abuse in Zambia: Focus on Training and the Development of a Forensic DNA Laboratory

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After attending this presentation, attendees will learn about the implementation and experiences of international multi-disciplinary collaborations and training to combat child sexual abuse with a focus on forensic DNA analysis, specifically on a developed and ongoing NGO fellowship training program, and share experiences of international professional exchanges between the U.S. and Zambia

This presentation will impact the forensic community by sharing the knowledge of international collaboration involving forensic science as one part of a multi-disciplinary approach to the complicated problem of stopping child sexual assault. It may help attendees start or improve their own forensic science related collaborations.

Georgian Foundation* and the Zambian Society for Child Protection** (both non-governmental organizations) have initiated a child centered multi-disciplinary program with the goal of assisting local communities in Zambia with the response to allegations of child abuse in ways that focus on the child victims. One objective is to create an effective system of inter-agency collaboration to respond to the numerous child sexual assault cases in and around Lusaka, the capital of Zambia. The program includes training in child advocacy, forensic and clinical medical examinations, police operations, forensic DNA analysis, and judicial prosecution.

To that end, an Experience Exchange Fellowship Program was created and is currently being coordinated and developed with participation of Zambian professionals across multiple disciplines. The Fellowship Program will enable healthcare, forensic, and police professionals from the Republic of Zambia to engage in practical specialty training programs in California and Utah. Police officials from Zambia have already had productive meetings and observational tours with law enforcement and forensic laboratories in the U.S. as well as attending a regional forensic science meeting in California. They also met with a District Attorney's Office prosecutor that manages investigation and prosecution of child sexual assault cases. Medical professionals from the United States with expertise in child protection have traveled to Zambia to assist with education and training of Zambian staff at the One Stop Center, a center for diagnosis and treatment of child sexual assault at the University Teaching Hospital in Lusaka.

In collaboration with the Center for Disease Control (CDC) and the University Teaching Hospital in Lusaka, the Fellowship Program will provide more intensive hands-on training experience for Zambian forensic science, police, and medical professionals. This phase of the training by forensic science professionals will include a thorough and documented forensic DNA analysis component that is expected to enhance the capacity of the participants to more effectively manage and coordinate interagency response in child sexual assault cases.

In addition to the Experience Exchange Fellowship Program, a main goal of the organization is to collaborate and support the Zambian government in establishing a forensic DNA laboratory in-country to facilitate prosecution in sexual assault cases. In the first steps toward this goal, medical and forensic science professionals from the United States have traveled to Zambia for strategic collaboration and planning with Zambian government officials.

Ultimately, the goal of the organization is to facilitate the development of an interagency working group including medical, police, forensic, and legal experts to strengthen the child sexual abuse response system in Zambia, thus reducing and preventing child sexual abuse and further transmission of HIV and AIDS.

*Georgian Foundation 501(c) 3 is an international organization registered in California, United States.

**Zambian Society for Child Protection (ZSCP) is a registered non-governmental organization founded by the Georgian Foundation.

Forensic DNA Lab, International Collaboration, Child Sexual Abuse

A222 Identification of Missing Mexican Nationals Along the U.S. — Mexico Border

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The goal of this presentation is to educate the forensic science community on collaboration efforts between private and public industries in the United States and Mexican authorities to identify missing migrants that have crossed the U.S. – Mexico border.

This presentation will impact the forensic science community by outlining a system of identification efforts involving communication with several entities to effectively provide closure to family members who are missing loved ones. The attendees will gain knowledge of the scope of missing migrants from Mexico believed to have perished in the U.S. and how relationships can be formed to aid in identification efforts in both the U.S. and worldwide.

Each year migrants from Mexico, as well as other parts of Central America, attempt to enter the U.S. by crossing the U.S. – Mexico border and traveling across the southwestern desert areas of the U.S. Many migrants die in the desert areas during this journey due to exposure to the elements and exhaustion, and their bodies are recovered by U.S. authorities. There are numerous reasons why these bodies are not identified and why there is a need for forensic DNA testing to confirm suspected identifications. An international system has been created in recent years to aid in the identification of the missing and to have their remains repatriated to their families in their native countries. As a vendor laboratory, Bode Technology (the laboratory) has been part of this international effort, in collaboration with both U.S. and Mexican authorities, to identify missing Mexican nationals whose bodies have been recovered in the desert southwestern areas of the United States.

Up to several hundred Mexican nationals perish in the desert southwestern areas of the U.S., with most of these deaths occurring in Arizona as well as some in other states. The Mexican Foreign Ministry has taken a high interest in the identification of missing Mexican nationals and has been working with U.S. officials to aid in their identification. Most of the recovered bodies are kept at the Pima County, Arizona, mortuary facility where skeletal cuttings are collected and sent to the laboratory for forensic DNA testing. The Mexican Foreign Ministry works primarily with U.S. consulates, especially the Mexican Consulate in Tucson, Arizona. They contact family members who are missing loved ones where the families believe the individual attempted to enter the U.S., and arrange for the collection of relevant family DNA reference samples. The family reference samples are then provided to the laboratory for testing and comparison to the DNA profiles from the skeletal remains to determine if a familial relationship exists. If a familial relationship is identified, a DNA match report is generated and sent to the Consulate.

There has been a great deal of success and experience in the DNA analysis of skeletal remains, and over the years special techniques have been developed to troubleshoot these challenging samples. The DNA testing typically performed is a standard forensic test that analyzes 15 nuclear STR loci and a gender-determining marker. To date, close to 400 skeletal samples and 200 family references have been tested, resulting in more than 80 identifications from the country of Mexico. The success rate in obtaining suitable STR DNA profiles from the skeletal remains has been more than 80%. On a few occasions, additional DNA testing, such as Y-STR and mini-STR testing, was required, usually due to limited availability of family references. After the DNA profiles are obtained, they are entered into a computer software program in order to calculate kinship statistics. In the cases where a potential familial

relationship was identified, the statistical calculations have generally yielded probabilities of family relationships of more than 99.95%, thus providing a high degree of confidence. This has been a highly successful system that is ongoing and is an example of how cross-border inter-governmental cooperation with private DNA providers can lead to closure for hundreds of family members.

Missing Persons, Human Identification, US-Mexico Border

A223 It's the Right Thing to Do — Virginia's Ground-Breaking Post-Conviction Program

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After attending this presentation, attendees will gain an appreciation for the logistics involved in identifying, analyzing, and reporting more than 750 post-conviction cases that involved 30-40 year-old cases in which original evidence was unavailable, existing samples were compromised, case information was incomplete, and DNA results were challenging.

This presentation will imact the forensic science community discussing Virginia's ground-breaking efforts to establish a program to inventory, test, and report on evidence in hundreds of post-conviction cases that were 30-40 years old.

In Virginia in the 1970s, an unorthodox habit believed to supplement testimony with demonstrative displays had a tremendous impact on the disposition of a number of these cases 30 to 40 years later. Despite the practice of local law enforcement agencies at that time to destroy evidence and case files for unsolved and adjudicated cases, a lead scientist at the agency and her trainees taped the swabs, cuttings, and threads used in conventional serological techniques into the corresponding worksheets for the case.

These worksheets became part of the respective case file and were kept at the facilities until they were sent to the Library of Virginia State Records Center. These documented transfers took place between the Department's custodian of records and a Records Center storage technician. It was at this point that the files were accessioned into the Library and kept in a documented location with limited entry, thus creating a chain of custody on the worksheets.

It wasn't until 1999, when a prosecutor conducted a routine inquiry for any remaining evidence retained at the Department in a particular case, that administrators saw these substrates taped into worksheets and considered them "evidence." Given that this was the only evidence remaining for the case in which the convicted offender requested DNA testing to prove his innocence, DNA polymerase chain reaction (PCR) testing was conducted using short tandem repeats (STRs) and not only demonstrated that the offender was not the source of the sexual assault evidence, but the DNA evidential profile was searched in the Virginia DNA Data Bank and a "hit" was obtained. Since there is no statute of limitations on rape in Virginia, these particular DNA results took on two roles: to support the innocence of the convicted individual, and to investigate and eventually prosecute the newly identified suspect.

Due to the success of subjecting this "taped-down" evidence to current DNA PCR techniques, then-Governor Mark Warner ordered testing in 31 sexual assault cases. The moderate success of obtaining DNA profiles from these previously compromised samples resulting in additional eliminations, some of which resulted in full pardons, led to the Governor's order in 2006 to test all appropriate cases between 1973 and 1988. In 1988, with the advent of blood-borne pathogens and accreditation, the "tape-down" practice ended. Of the 530,000 case files generated in the Department by all disciplines during that time span, over 2,100 were found to contain biological evidence taped into the case files. In order to be able to track these files created prior to the computer era, a database was created which could be updated as cases were identified, original case dispositions were determined, evidence was tested, and results were reported.

This was the beginning of a ground-breaking post-conviction testing program for Virginia. Six years into the project, over 750 cases in which convictions could be confirmed have been analyzed and countless hours have been spent by a core group of scientists and administrators who agree with Governor Warner that "a look back at these retained case files is the only morally acceptable course, and what truth they can bring only bolsters confidence in our system."

The scope of this unique project with the challenges accompanying it was unprecedented. Case files lacked essential details usually provided in current cases and were not available through the submitting agency; in fact, given the dates of these cases, the majority of the original law enforcement officials had since retired. Samples consisting of threads, swabs, or cuttings previously subjected to multiple saline washes as part of ABO blood and secretion testing resulted in partial DNA profiles (or no DNA profiles). The level of interpretation difficulty increased due to both the absence of associated known sample(s) and the lack of DNA results developed from those which were preserved. These challenges, in addition to a case study demonstrating the obstacles encountered by both scientists and prosecutors when preparing a post-conviction case for prosecution of a newly-identified suspect, will be discussed.

DNA, Post-Conviction, Virginia

A224 A Decade of Research on the Genetic Identification of the Manufacturers of Improvised Explosive Devices

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After attending this presentation, attendees will become familiar with the large amount of research that has been conducted at Michigan State University during the past ten years on post-deflagration genetic identification of individuals who manufacture IEDs, including tests of the device itself, trigger components, and containers used to conceal the device.

This presentation will impact the forensic science community by presenting an overview of the large amount of DNA-based research that has been conducted in a laboratory on identifying those who make IEDs. Both nuclear and mitochondrial DNA testing from deflagrated pipe bombs will be covered, as will post-blast testing of items associated with IEDs, including trigger components and packaging. The presentation will summarize the most effective methodologies for isolating and analyzing DNA from this type of forensic evidence.

Improvised explosive devices have become an integral weapon for terrorists, both inside and outside the United States. IEDs are generally simple to manufacture and conceal, often being made from components easily obtained from hardware and sporting good stores, and hidden in bags or backpacks. During the blast, much of the potential forensic evidence is destroyed, including fingerprints, thus only class evidence is usually recovered, such as the type of explosive used and the hardware of the device. This evidence may have value if its origin can be traced to a specific location (e.g., a store where it was purchased), but, in general, it does not lead to the identification of the perpetrator.

For the past decade, forensic biologists at Michigan State University, in collaboration with the Michigan State Police Bomb Squad, have studied genetic methods for identifying manufacturers of IEDs, based on the premise that even if fingerprints are destroyed on a deflagrated device, enough residual cellular material may remain that an identification is possible. The first such study used standard forensic DNA techniques on pipe bomb fragments and resulted in a few alleles consistent with an assembler. MtDNA was next tested, and produced a much higher rate of identification, based on a closed population of individuals. New assays of deflagrated IEDs, including both NIST-produced and commercial miniSTRs, showed greater sensitivity than did standard STRs. The methods for retrieving DNA from deflagrated IEDs, which can be fragmented into thousands of small pieces, were assessed by comparing swabbing and soaking both deflagrated PVC and steel bombs. Similarly, positive and negative influences of cyanoacrylate fuming IED fragments were examined.

Materials associated with IEDs have also been extensively tested, based on the idea that these may be handled longer than the device itself, and are more detached from the device, so they might maintain cells/DNA better. Mock electronic triggers made up of a communication device (cell phone or walkie-talkie), battery, circuit board, and wiring, were placed adjacent to IEDs during deflagration, and the components was assayed using miniSTRs. Genetic data from each individual component were analyzed, as were data from all components *in toto*.

Likewise, IED containers were tested post-deflagration. Backpacks were used for several days, then bombs were placed inside and deflagrated. Multiple regions recovered from a backpack (e.g., zippers, straps, canvas) were tested via miniSTRs, and individual and combined results were used to identify a wearer.

The ability to analyze STR data solely or in combination with other data from the same object (e.g., components of a triggering device or parts of a backpack) helped differentiate "consensus profiles" from those that might contain rare drop-in alleles or mixtures. In most instances, consensus profiling resulted in far more accurate data for the IED/component handler than did STR results from individual objects.

Altogether, it is clear that genetic evidence can be obtained from deflagrated IEDs and the components associated with those weapons. However, such testing, while very sensitive, is dominated by low copy number and highly degraded DNA. As such, it can potentially be lost or compromised during evidence collection and processing, is susceptible to external contamination, and is affected by methods of DNA isolation, testing, and data analysis.

DNA, IED, Touch DNA

B1 A Forensic Comparison of NTFS and FAT32 File Systems

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After attending this presentation, attendees will: (1) understand the fundamental differences between the New Technology File System (NTFS) and the file allocation table 32-bit file system (FAT32); (2) gain an understanding of basic digital forensic knowledge of these file systems and the complications and advantages of each.

This presentation will impact the forensic science community by recognizing the importance of understanding the foundation of a science before anything more can be studied, stressing that the organization and processes of file systems must be understood. This research works to bring foundational data into one coherent discussion so that one can accurately assess advantages and disadvantages of specific file system forensic analysis.

The file system in any storage device is essential to the overall organization, storage mechanisms, and data control of the device. A file system can be thought of as an index in a book, where the book can be broken down into sections and chapters. Without this breakdown of sections and chapters in a book, it would be harder to find the information that is being searched for.

The same principles apply to file systems on a computer or storage device.[1] File systems utilize hierarchical structures to organize files and file directories into useable formats. This formatting is accomplished through the use of clusters, sectors, and data entries located on a hard disk. Within data entries are located metadata files, which store user and application data about file dates and times, length, file size, file names, and more.[2] Knowing how these file systems work and the layout of key structures, storage mechanisms, associated metadata, and file system characteristics is essential to being able to forensically investigate a computer or other device.

The focus of this research is to differentiate and compare the two file systems, New Technology File System (NTFS) and File Allocation Table (FAT32), in eight areas. The eight areas are: key structures, storage mechanisms, file names, directories, file date and time, file deletion, encryption, and forensic implications.

NTFS is a newer file system, beginning with Windows® NT and 2000, and has brought in a lot of new features, including better metadata support and advanced data structures.[3] FAT systems were originally used in DOS and Windows versions prior to Windows XP. The 32 in FAT refers to the 32-bit numbers that represent the cluster values. Even though the FAT operating system is not utilized in many newer hard drives, it is still often used as a default file system in removable media and storage devices, as well as computers with multiple operating systems.[4]

One of the major differences between the NTFS and FAT32 file systems is that NTFS uses a Master File Table (MFT) and FAT32 uses a File Allocation Table (FAT).[4,5] The MFT table houses data entries for all files. Even metadata files have an entry in the MFT table. NTFS stores all of its data in attributes, which are simply data files. One attribute will house metadata, one will house file data, like size, and one will store the actual file content.[5,6]

There are many more attributes than this. The FAT table simply points to where the file is housed within the file system. Any metadata is stored in the header at the beginning of the file.[1] Some other noted differences are the process of file naming, where FAT utilizes both long and short (8.3) names (8.3 filename, or 255 UTF-16 characters when using LFN) and that NTFS uses long file names (255 UTF-16 code units).[4] Other differences noted are with the file deletion and encryption processes. NTFS was designed with fairly advanced security, whereas FAT32 has little to no encryption capability and was not designed with security in mind.[7,8]

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NTFS, FAT32, File Systems

B2 A Narratological Approach to Digital Forensic Analysis Utilizing Natural Language Toolkit

Mark Pollitt, MS, Daytona State College, 1770 Technology Blvd, Daytona Beach, FL 32117*

After attending this presentation, attendees will gain an understanding of how narratology and natural language processing can be applied to the analysis of digital evidence.

This presentation will impact the forensic science community by providing results of initial experimentation with the application of the theory of narratology, coupled with the Natural Language Toolkit (NLTK), to a forensic corpus.

Digital forensics has made tremendous strides in the acquisition and preservation of electronically stored evidence. As stored data has grown ever larger and more complex, the ability to identify files and data that are investigatively significant and legally probative has not kept pace with this growth. The traditional approach to digital forensics has relied upon the use of metadata such as file system dates and times, classification of files by data type, and simple string searches. With the advent of ever larger storage media, the ability of digital forensic examiners to identify information of investigative or probative value has become less efficient. In earlier work the author has described the concept of narrative as relates to the search for meaning in digital evidence.[1]

Narratology has been defined as: "the ensemble of theories of narratives, narrative texts, images, spectacles, events; cultural artifacts that "tell a story."[2] Digital forensics seek to identify and tell the investigative "story" as it relates to the case under investigation. One

part of the narratological theory defines the elements that make up a narrative, such as, actors, events, and chronology. If textual material can be processed in such a way as to identify these elements, then it may be possible to extract the "story" from the text and, by extension, approximate the probative "story." This presentation will explore how this theory can be used to develop criteria for the automated processing of textual material to increase its investigative value, and how using natural language processing tools could be used to assist in the identification of these elements.

In order to test this hypothetical approach, a series of experiments, utilizing the Enron email corpus will be conducted, using a number of procedures included in the Natural Language Toolkit.[3,4] This corpus is a collection of the actual emails sent by employees in the criminal trial of several Enron employees that was admitted into evidence during their trial and was subsequently released to the public by the courts. This corpus was selected since the data is in textual format, the metadata from the email headers has been converted to text, and the "story" is known. The results of these experiments will be reported and an assessment of their value to digital forensic examination will be made.

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Narratology, Language Processing, NLTK

B3 Linking Persons Based on PRNU on Social Network Sites

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The goal of this presentation is to learn possibilities and limitations of camera identification based on Photo Response Non-Uniformity (PRNU).

This presentation will impact the forensic science community by offering new methods to link persons on spoofed identities as a result of the massive expansion of social network sites and data it leaves behind.

Social media websites such as Facebook®, Flickr®, and YouTube® contain large numbers of photo and video material.

Photos and videos from databases, such as a child pornography database, can be linked with these social media websites, in order to find a potential suspect. It is also possible to determine if a suspect camera took certain images when a suspect camera is available. The linking of photos and videos is done by making use of the PRNU pattern.

A digital (video) camera consists of many electronic components. After the image has been formed on the image sensor, the image information will pass through all of the components before the final data file is written to flash memory. Each step in this process may add random noise to the image. Even during the image formation process itself, a noise-like pattern from the sensor may be introduced in the image. This noise-like pattern is a small but measurable systematic contribution to the signal, and is called the PRNU pattern. It is caused by small variations of pixel sensitivity to light. The visibility of this signal is limited, and may be a small difference depending on the intensity of the signal. In practice, this means that well-illuminated images will result in a better extraction of this signal compared to when the image is dark. The PRNU pattern itself can be determined from the image and is preferably done with images that have no discernible textures (flat field image for example, from a grey surface). Research so far seems to indicate that every camera has its own unique PRNU pattern. The examining of the PRNU pattern for forensic use is well researched by Jessica Fridrich and others.

The best situation would be to have the suspect camera so flat field images can be made. In practice, it is not always possible to have the camera for casework, such as when linking images in databases to social media websites. However, it is possible to determine if a set of images has been made with the same camera or a different camera based on the PRNU pattern. When the camera is available, the pattern from a questioned image can be compared with the pattern from a set of reference images made with a suspect camera. It can be determined whether the questioned image was produced with the suspect camera or not. This works when the image is authentic, but fails when the image underwent any spatial transformations (e.g., rotation, shearing, resizing) because the "fingerprint" is desynchronized, unless the same transformations are applied to the reference material. It is also possible to alter the image such that the PRNU pattern is filtered out, although this is complicated and time consuming.

Linking large image or video databases requires significant processing power and time. For a good comparison, it is important to have untampered, original images, or to know exactly what kind of operations have been conducted on the image. Since in casework the ground truth is not known, conclusions are given in a Bayesian framework.

In this presentation, an overview will be given of methods that can be used for faster calculation by using only a part of an image, as well as solutions for when the camera is not working by changing the camera module. Also, the possibilities of examining images and videostreams from social networks such as YouTube® and Facebook® are discussed.

Camera Identification, PRNU, Social Networks

B4 StealthBots: A Digital Forensics Case Study

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After attending the presentation, the attendees will be able to understand the impact that stealthbots have on digital investigations, detect footprints of a stealthbot in the file system, and explain the defense strategy that is being proffered regarding stealthbots.

This presentation will impact the forensic science community by highlighting the challenges of investigations where the stealthbot defense, almost impossible to disprove at this time, is used.

As the legal justice system becomes better educated and mature in the domain of digital investigations, standard defense strategies are appearing. One such strategy is to blame someone else. A more sophisticated expansion on this legal defense is that while the system belonging to the accused was used to perpetrate the crime, the accused was not the one sitting behind the keyboard, or was "framed" by some unknown entity. To date this defense has met with minimal success. With the introduction of stealth malware such as the Zeus bot Stealth (Zbot-Stealth) into the "wild," this defense takes on a entirely new dimension.

The Zbots have been primarily used by the criminal community to attack online banking systems. These bots have become increasingly sophisticated and can be modified to attack particular banks. The bots are "made to hire" and can be purchased online from the developers for prices ranging from $2,000 to $20,000 USD. The complex nature of these "made to order" malware programs has made the detection and investigation of cases more difficult. However, these bots tended to leave "footprints" in file systems that could be discovered and readily identified as malware. The anti-virus industry has also developed detection and removal capabilities related specifically to bots.

Recently, a new variant of the Zbot was released that has been coded to implement stealth and anti-forensics. The anti-forensics capabilities, while not technically new, allow the program to remove its traces from the infected system and obfuscate any remnants showing it was ever installed. The Zbot-stealth variants appear to be very effective at erasing all traces in the file system that they were ever installed and/or

executed on the infected system. The real novelty of the stealth variant is its ability to take control of the victim's system at the same time that the victim is still logged in and using the system. This is very unique. Prior variants relied on the standard Remote Desktop Protocol (RDP) and Virtual Network Computing (VNC) to gain access and control of the victim's system. Using the standard method of RDP, the system would terminate the access of any user logged on locally and hand over control to the remote user. This meant that the victim would be able to detect that a remote control session had occurred. The new variants have modified or "hacked" the RDP protocol and now control of the system can be gained remotely without the local logged-in user being logged-out. Now the attacker can easily "piggyback" on the victim's activity and orchestrate an attack from the victim's system with the victim still using the system. The resulting traces of the attack and the victim's normal system usage behavior become co-mingled and extremely difficult to differentiate.

The presentation will discuss the technical aspects of the Zbot-Stealth variant, the forensic challenges associated with the new attack vector used by the malware, and how to effectively examine and analyze a system that may have been infected. A case study will briefly be introduced where the Zbot-stealth defense was put forward. The case study highlights the difficulty in countering this defense given the lack of positive forensic evidence left in the digital crime scene by this new class of attacks.

Bots, Stealth, Digital

B5 Forgotten Forensics: Understanding Risk, Data Loss Prevention, and Incident Forensics in Cases of Unauthorized Access to Dial-In Conference Calling Lines and Voicemail

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After attending this session, attendees will understand the risks associated with dial-in conference lines and voicemail systems, understand cases in which they have been misused, legal implications of such misuse, how to conduct a forensic analysis of these systems, and preventive measures to minimize misuse of these facilities.

This presentation will impact the forensic science community by focusing on areas of digital telephony that are often overlooked by investigators and forensic scientists looking into data loss situations. In the past year, at least one major case involving interception of a call between U.S. and U.K. law enforcement personnel gained a great deal of publicity. Similarly, abuse of voicemail systems by reporters involving both crime victims and celebrities, have shaken the U.K. newspaper industry. This presentation will provide a good basis for understanding and pursuing investigations involving dial-in telephone conference calls and voicemail systems.

In 2012, members of "Anonymous" posted a recording of a conference call between U.S. and U.K. law enforcement officials. While the unauthorized party joined the call and, in fact, recorded it, none of the authorized participants knew this was happening. In another case, an employee who left one employer to join a competitor simply continued, after changing jobs, to dial into regularly-scheduled but highly confidential calls run by the original employer which provided him with valuable competitive information. Both cases resulted in legal action against the unauthorized users. It is believed; however, that conference call bridges as a source of data leakage are not generally thought about by digital investigators or by specialists in the digital and multimedia forensic sciences and often lack even the simplest defensive measures.

In addition, the break-ins to the voicemail accounts of crime victims and celebrities by U.K.-based reporters reminds us that these systems are in almost universal use in both home and business environments, and are often only protected by no more than an easily guessed password. With many systems offering a "mark as unheard" functionality, the potential for abuse is obvious.

In this presentation, attendees will be shown cases in which unauthorized access to dial-in conference call lines (sometimes called "call bridges") and voicemail systems resulted in the loss of highly proprietary, personal, sensitive, and confidential data. The major types of call-bridge and voicemail services will be described, and some of the forensic data that can be available either during a call or in a post-call investigation will be demonstrated. With this study, it is believed that these services are often overlooked when assessing risk and seeking the source of a data loss incident.

Questions to be answered during this session include:

- How are conference-calling lines and voicemail systems abused?
- How do unauthorized persons gain access to these lines?
- Can they also use the conference line for unauthorized calls at the company's expense?
- What investigative and forensic data is available, and how can it be obtained?
- What are the legal implications of this abuse?
- How can conference-call and voicemail users prevent problems, before, during, and after use of these lines?
- Who is responsible for the investigation of cases of this type?

This presentation is suitable for digital and multimedia specialists as well as lawyers, as there are important legal and procedural safeguards that should be considered, particularly in the case of calls involving privileged or highly sensitive information.

The presentation will begin with laying out the technology and the issues. This will be followed by some case studies, and then by both a legal and forensic science review of the problem, investigative guidelines, and ways to prevent misuse or abuse of these important business tools. Ample time will be provided for audience questions and answers and to share participant experience interactively. Participants will be provided with a sample guide for conference call users to enable them to minimize the likelihood of such problems occurring.

Voicemail, Conference-Line, Telephony

B6 3D Morphometric Computer-Assisted Comparison of Faces, Hands, and Other Body Features in Databases

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The goal of this presentation is to provide a methodology for comparison, using Likelihood Ratios (LRs) for the comparison in addition to the use of reconstruction images, limitations, and possibilities.

This presentation will impact the forensic science community by showing how to combine conclusions of biometric features with a Bayesian framework.

In forensic image analysis, the question is often asked if the person on a certain image (e.g., from CCTV, documents, child porn) is the same as the suspect. Sometimes only a part of a body is visible, so only parts of a body are compared with the suspect.

The method used for comparison of objects and of humans is to position the person or object in the same position as the questioned image. If the camera system is not available, a 3D image will be made of the person, such that the 3D image can be positioned in the same way. For facial comparison, there is a methodology for comparison which is used in different scenes.

On recordings of certain crimes, the face is not always (properly) shown, unlike the hands. Therefore, the need has arisen to develop a method for the identification of people based on their hands. The research method that is applied for hand comparison is based on morphology, anthropometry, and biometry of hands. A manual test (checklist) was created which includes characteristics of both the back of the hand (e.g., the subcutaneous vascular pattern in the back of the

hand) and the palm of the hand (e.g., palm line pattern). Features that comprise both parts of the hand are also included (e.g., proportions and typica). To assess the location of pigmented lesions, the hand can be segmented into 14 regions using readily discernible anatomical landmarks. Then, each hand can be assessed for the number of features found in each area. Furthermore, a database is available that not only contains many hands, but also scripts that can compare images and provide identification rates. Initially, the scripts were only capable of identifying the palms of the hands. Recently, they are also capable of analyzing the backs of the hands.

For other parts of the body, such as the abdomen and genitals, there is no structured approach known to the authors; however, similar methods can be used. In casework, this study focused on different features of the body, where the skin characteristics as well as the shapes were used. The reference images of the body are taken by a forensic physician. During the comparison, different features are compared manually and the investigator will provide information on apparent similarities and differences which are further evaluated by classifying features as:

1. WD - Weakly discriminating, e.g., shape and size of body parts.
2. MD - Moderately discriminating, e.g., lines and position of veins.
3. SD - Strongly discriminating, e.g., the shape and position of a scar or pigmentation.

The comparison should preferably be performed by three independent investigators. The lists are combined after careful deliberation by the investigators into the list of observations added to the report. This list of observations can be used to check the reported comparison results and conclusions.

Concerning the forensic hand comparison, it has been tested to ascertain whether the manual or automated method performs better when analyzing hands from the database. Currently, it is being investigated to determine if the tests work with "homemade" photos as well. Eventually, the aim is to investigate hand images made by CCTV systems, which are often of very poor quality. There is a need for an automated comparison and a more statistical approach for this kind of research. Large databases with standardized image material could be helpful in having a Bayesian approach in this biometric comparison. Since it is not yet perfected for facial comparison, it is expected that, in the meantime, the visual comparison based on features and describing the differences and the similarities will be an important approach for this type of examination.

Facial Comparison, Hands, Other Body Features

B7 Establishing Criteria for the Authentication of Digital Imagery

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After attending this presentation, attendees will understand an analytical approach to determine if a digital image contains traces of manipulation, combining different manipulation-detecting techniques relevant to image authentication. The cognitive outline will help attendees evaluate their findings when making decisions concerning the authenticity of digital image files.

This presentation will impact the forensic science community by strengthening the criteria needed for authenticating digital images.

Digital photography has replaced film-based photography as the prominent means of acquiring images. The ability to create digital images has been integrated into cameras, cell phones, scanners, and other devices. Due to the widespread availability of image processing tools, it has become increasingly simple to modify digital image content with no perceivable indications of alteration to the naked human eye alone. When digital images are central in an investigation or produced as evidence, it may be important to verify the image files' authenticity and/or integrity. Opening, processing, and resaving a photo utilizing image processing software will create modifications in an image file. In addition, applying anti-forensic techniques to obfuscate manipulation leaves detectable traces. While there exists a wide range of manipulation detection techniques, the simple fact is that an individual with the proper tools, knowledge, and skill can create forgeries that can elude detection by any one of these techniques. However, while certain manipulation techniques can elude one or more analyses, it is difficult to elude them all.

This presentation proposes a robust authentication framework that incorporates strong manipulation detecting techniques, collectively focusing on as many unique aspects of the digital image file as possible to diminish the possibility that traces of manipulation go undetected by image analysts. Analyses of the digital file concentrate on the binary data that comprises the information about the file container, including the file format, HEX data, EXIF, MAC stamps, and file marker analysis to help uncover clear signs and traces of a digital image file's history. Global image analysis techniques examine the information that represents the overall configuration of the image content where modifications can alter the random distribution of pixel values in original images and introduce mathematical relationships not found in original, undoctored images. When analyzing the global structure, techniques focus on compression schemes, interpolation, the color filter array, RGB color distributions, quantization tables, error level analysis, and DCT coefficients. Techniques that examine the statistical relationships that exist between neighboring pixels, such as copy and paste detection, photo-response non-uniformity comparisons, correlation or probability maps, histogram equalization, and JPEG error level analysis, are included in the analysis of the local image structure. Additionally, source image identification exploits small imperfections in the image sensor, such as its Photo-Response Non-Uniformity, where pixel defects are imprinted onto the output image file consistently occurring from one image taken by the camera to the next. These irregularities can be used to determine if the image in question could have come from a specific acquisition device.

By combining techniques that examine visual image inconsistencies with different areas of a digital image structure, the probability that manipulations in a digital image will go undetected are greatly reduced. To assure quality in image authentication examinations, cross-verifying conclusive findings using a minimum of two different methods is recommended. Furthermore, when employing automated algorithms, it is necessary to verify their performance on realistic databases and image manipulation techniques before applying them in real cases. It is important to note that these techniques can only provide positive proof of image tampering. It is extremely difficult, or even impossible, to prove that an image is free of modification; the best that an analyst can do when authenticating digital images is to search for indications that support the hypothesis that the image was generated without modification. In addition to inconclusive findings, the conclusions an expert can reach shall be that the evidence is either consistent or inconsistent with an original image.

Media Forensics, Image Authentication, Media Authentication

B8 Establishing Likelihood Ratios for Patterned Garment Comparisons From Seam Measurement Data

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After attending this presentation, attendees will understand how common garment manufacturing processes can support variations in the appearance of patterned garments, and how these variations can be

measured and exploited to calculate a conservative estimate of the probability of an accidental match between two random garments.

This presentation will impact the forensic science community by establishing a methodology to assign quantitative measures of evidence strength to patterned garment comparisons.

Surveillance images of a crime scene present a potential wealth of evidence that may be used to facilitate identification of objects relevant to an investigation; however, it is often a challenge to ascribe an objective metric of confidence for a particular identification. In the case of patterned garments, an apparent match between the item seized from a suspect and the one captured in the surveillance images of the crime scene can represent a compelling piece of evidence, if properly quantified. This work develops a rigorous statistical model for estimating such quantitative metric—the Likelihood Ratio (LR)—for this class of forensic comparisons.

A visual match between two patterned garments is established if the pattern offsets at the seams of all the visible individual pieces are sufficiently similar, i.e., all the differences can be explained by the limited precision of the measurements on the surveillance image data. Accordingly, the probability that the match is accidental (and hence its likelihood ratio) depends on the quality and resolution of the surveillance imagery, the number of visible garment seams/pieces, and the joint statistical distribution of pattern offsets of the observed garment pieces. The developed model provides a methodology to measure or estimate these parameters. Upper-bound validity is maintained throughout the model ensuring that the probability of accidental matches is not underestimated and LR not overestimated. The model builds on previous work by the authors which developed similar methods for camouflage garments based on direct measurement of the manufacturing process parameters. The new approach extends applicability to all patterned garments and greatly simplifies the model and its practical implementation by not requiring access to the manufacturing facilities. Instead, a limited set of the same garments is acquired and the statistical distributions and dependence of the pattern offsets for different garment pieces are evaluated empirically. Model simplifications and empirical parameter estimation result in larger uncertainties and, hence, somewhat lower bounds on LR values. This can be partially compensated by increasing the sample size: while not affecting the unbiased LR estimates, larger samples reduce the confidence intervals and thus generate tighter upper and lower bounds for probabilities and LR, respectively.

The model was demonstrated and validated through a large scale empirical study with simulated automatic garment comparisons and manual image comparisons. An empirical study of 12 garment sets, each with 25 to 50 samples, yielded LRs ranging from roughly 10-to-1 to over 400-to-1, i.e., from weak and inconclusive to strong evidence. In all test cases, accidental match probabilities were lower than the upper-bound model predictions, confirming that the developed framework produces conservative and defensible lower-bound LR estimates. A software tool was developed and is freely available to assist examiners in executing studies in this area.

Individualization, Patterned Garments, Statistics

B9 A Case Study in 3GP File Analysis and Repair

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After attending this presentation, attendees will understand some principles in digital data analysis, multimedia file structure and repair, and how to use various software tools to reconstruct multimedia metadata. Attendees will learn the relationship between recorded data and multimedia containers revealing the usefulness of Hex data analysis and the reverse engineering of file formats.

This presentation will impact the forensic science community by discussing a real case involving the analysis and repair of a corrupt 3GP file recorded by a cell phone. An overview of the QuickTime file format specification will be provided along with comparisons to various other format specifications. Hex data analysis of recorded media will be demonstrated, highlighting things to look for when analyzing file formats for repair.

Corrupt or incomplete media files are a common problem encountered during the course of investigations. In these cases, the recorded audio and/or video material may not be playable and, therefore, important information related to a case is irretrievable. File corruption may be due to any one of several reasons, such as: hardware malfunction resulting in the file not properly being closed or wrapped following the recording process, incomplete fragments of recordings were detected and carved from physical media during the course of data imaging and analysis, deletion and overwriting of media files have rendered them corrupt, etc. However, if a forensic media analyst is resourceful, the data structure of the file may be reverse engineered and repaired making playback possible, revealing the otherwise irretrievable recorded contents. In these cases, resourcefulness is the key because the process of file repair depends on the format and codec of the recording, and not all manufacturers implement technical specifications in the same way. This means that two 3GP files, for instance, recorded on different equipment will require different procedures for repair due to inconsistently structured data. When the organization of a media file's data structure is determined, analysis and repair may be possible by referring to available file format specifications and by using Hex viewing/editing software that displays a file's binary data in hexadecimal notation and ASCII text. Last, a software tool for reconstructing large amounts of data may be necessary where manual creation of Hex data is not possible. Once a file has been repaired, investigative review can take place, or further forensic analysis is made possible.

Following the presentation of this background information, technical details regarding the analysis, repair, and reconstruction of the recovered 3GP file in the case study which resulted in making a broken file playable will be discussed. The method used to repair the file is comprised of: hex data comparison of the evidence file to playable exemplar files recorded by the same phone, identification of the missing and inaccurate metadata that made playback not possible, reconstruction of the missing Hex data using Matlab software, and amendment of the corrupt file to make playback possible.

Multimedia Forensics, Audio Forensics, Hex Analysis

B10 Live Forensic Analysis of Kernel Code for Malware Detection in Cloud Computing Environments

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After attending this presentation, attendees will understand key characteristics of cloud computing environments, including typical uses of virtualization and how virtualization can be used to support live forensic analysis of operating system kernel code for malware detection. Existing techniques to assess the health of kernel code require pre-computing values such as cryptographic hashes, which is problematic in the face of dynamic updates and multiple kernel versions. A technique that leverages virtual machine introspection to assess kernel code health at runtime without relying on pre-computed values will be illustrated.

This presentation will impact the forensic science community by discussing a method to perform correlation of kernel code running in multiple virtual machines in a cloud environment without relying on traditional techniques, such as cryptographic hashes. This work is important because developing and maintaining pre-computed values for kernel code components for a large number of operating systems and kernel versions is cumbersome and has a negative impact on performance.

The integrity of operating system kernel code needs to be maintained in order to avoid disruption of service and to prevent malware from either interfering with normal system operation or accessing

sensitive information. Kernel code integrity is usually violated by either patching the kernel with malicious code or loading malicious kernel modules/device drivers. Before corrective actions such as snapshot restoration can take place, malicious kernel tampering must first be detected. State-of-the-art solutions typically use a dictionary of hashes of kernel code or malware signatures for detecting malware infection. In the case of cryptographic hashes, the hashes for key "known good" kernel components are pre-computed and these values are periodically compared with the hashes of code currently loaded in the kernel. Any discrepancy indicates a potential infection.

The key problem in maintaining hashes is that code changes across different kernel versions of an operating system. Even kernel patches (e.g., for removing bugs and security vulnerabilities) change the kernel code. Thus, the dictionary needs to be maintained for all kernel versions and all the patches for each kernel version, which is a time-consuming and cumbersome task. Also, the dictionary needs to be upgraded constantly to accommodate new patches and kernel versions in order to ensure effective malware detection. Moreover, if the dictionary needs to be maintained for a cloud server where several different operating systems are run on tens or hundreds of virtual machines at a time, the scale of the problem increases significantly, requiring maintaining the dictionary for all the operating systems in the cloud server.

Recent work to automatically perform live forensic analysis of kernel code at runtime in a cloud server without maintaining a dictionary of hashes will be presented. The work uses a typical scenario in a cloud server where a pool of virtual machines runs an identical kernel version for a guest operating system. Such pools of virtual machines are maintained to simplify the maintenance process so that applying patches and upgrading systems can be automated. The technique in the presented work compares the in-memory kernel code (including kernel modules) of all the virtual machines in a pool and is based on the assumption that if kernel code has not been altered, it should be the same across all the virtual machines and any discrepancy potentially indicates a malware infection. When discrepancies are noted, additional steps such as gathering snapshots for further forensic investigation and restoring the system to a known good state can be undertaken.

A prototype implementation of the technique will be discussed in the presentation. This tool runs on a privileged virtual machine where it can access the physical memory of other virtual machines in the pool through virtual machine introspection. This technique is resistant to tampering by malware because no components run inside the guest virtual machines. This prototype is for Microsoft® Windows® and the technique has been tested against a variety of exploits, including those based on opcode replacement and DLL hooking. This technique successfully detects all of the tested exploits and does not affect the performance of the guest operating systems.

Digital Forensics, Code Integrity, Cloud Computing

B11 Personal Identification by Superimposition and Metrical Analysis: Practical Experience

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After attending this presentation, attendees will better understand the technique of personal identification by morphometric comparison between recorded images of a robbery and 3D photogrammetric facial avatars of the suspect.

This presentation will impact the forensic science community by showing an objective, repeatable, and non-invasive method for robber personal identification based on facial superimposition and facial landmark metrical analysis.

Among the available techniques for personal identification—morphological classification, metrical analysis, and facial superimposition—the latter seems to be the most reliable; however, very often courts require a quantification of the correspondence between recorded images and a robber's face.

The described method exploits digital photogrammetry for facial scanning. Photogrammetric 3D capturing works by multiple and synchronous photo-shooting and represents the most suitable technology for face capturing in living persons, while laser scanning is largely affected by slight movements of human faces.

In January 2010, two criminals armed with paper cutters, burglarized a bank in Bari. Their faces were covered by balaclavas when they entered the bank; however, a small camera was hidden in the bank security entrance doorpost and partially captured the robbers' faces before they were disguised.

Some months later, police officers recognized the two robbers as two local criminals who were already spending time in jail for similar offenses. The judge authorized the research group to go to the jail for the 3D acquisition phase. In order to do this, the suspects' authorization was requested and obtained.

The equipment transported to the prison consisted of four cameras (12.1 Mpixel), a proper support, the lighting system, a calibration grid, and a notebook for the synchronization of photo-shooting and data storage. The suspect 3D avatars were created with a suitable software program and were then saved as ".obj" files.

In the next step, recorded images of the robbery were carefully analyzed and only frames with a better view of facial landmarks were chosen for the 2D/3D comparison.

The hidden camera had a fisheye lens and, to proceed to the superimposition phase, fisheye distortion removal was needed. To do this, an on-site inspection of the bank entrance facilities was carried out, with accurate measurements taken of the bank entrance room furniture and walls. The selected frames were then normalized through comparison with the obtained data.

The 3D avatars of the two suspects were then spatially oriented in the same position as the robbers in the normalized frames. To check the correct spatial orientation, the 2D frames and the snapshots of the 3D avatars were superimposed and gradually blended.

In the next step, for each face and avatar at least five objective landmarks were chosen (both in lateral and in frontal view), such as glabella, exocanthions, endocanthions, pronasal point, subspinal point, and gnathion and pogonion. Repeated landmarking by different observers allowed detection of random errors and controlled the quality of the landmarking within and between operators, minimizing variability.

Finally, the absolute and relative distances between the marked points, the perimeters and the areas of the triangles obtained by connecting the points, and the compactness indexes were automatically calculated on both images in the analysis using suitable software. The correlation coefficients between the suspects' avatars and the robbers' faces values that were higher than 0.995 were judged to be consistent with a positive personal identification.

Identification, Robber, 3D

B12 A Digital Forensic Analysis on the iCloud® and Its Synchronization to Apple® Devices

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After attending this presentation, attendees will know what iCloud® artifacts can be found on the iPod Touch 4G® and the MacBook Pro®. Additionally, the audience will learn one way to determine if two Apple devices are synced together through the iCloud®.

This presentation will impact the forensic science community by showing preliminary steps of how to capture artifacts from iCloud®-enabled devices.

In October 2011, Steve Jobs introduced the iCloud® to Apple customers.[1] The iCloud® was a service that allowed Apple device users to sync various applications to remote servers and access the data from each of their Apple products.[2] The study of cloud computing services,

like the iCloud®, is a burgeoning area of research for the digital forensic community. The goal of this project was to show artifacts that confirmed iCloud® activation. Since the iCloud® is not a physical device that an investigator can seize, it is important for forensic examiners to know how to determine if a device is iCloud®-enabled.[3] Additionally, if multiple devices were connected to the iCloud®, there could have been residual artifacts that showed a link between the devices.

The iPod Touch® and MacBook Pro® were tested because their operating systems, iOS 5.0.1 and Mac OS X 10.7 Lion, respectively, were equipped to utilize the iCloud®, and were supported by current forensic tools. Three images were taken of the iPod Touch®: (1) before iTunes and iCloud® activation; (2) after iTunes and before iCloud® activation; and, (3) after iTunes and iCloud® activation. Two images were made of the MacBook's® solid state drive: before and after iCloud® activation. A comparison and analysis of the images were then performed to identify artifacts resulting from the enabling and use of the iCloud®.

On the iPod Touch® and MacBook Pro®, property lists (plists) differed between images created both before and after enabling the iCloud®. Certain dates and key values that were found to support the iCloud® were enabled on the iPod Touch® and MacBook Pro®. However, little evidence was found to show both devices were clearly connected to each other via iCloud®. The evidence that was identified came from the synced applications that were linked to the iCloud®. Data from certain applications were displayed on both devices, which supported the theory that the data was synchronized.

Therefore, it is possible to obtain iCloud® information from the local drives of iDevices. Further research must be done to determine the synchronization of information to and from the iCloud®. A subsequent step to take would be to attempt to monitor iCloud® traffic between Apple devices. Also, a protocol should be written on a standard way to capture the iCloud®, as well as create live image tools.

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iCloud®, Apple®, Synchronization

B13 Forensic Analysis of Twitter® Artifacts Using the Twitter® Web Interface and TweetDeck®

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After attending this presentation, attendees will have an understanding of types of artifacts left behind on a computer running the Windows operating system using the Twitter® web interface and using the popular TweetDeck® desktop software offered by Twitter®.

This presentation will impact the forensic science community by providing information to assist in the identification of artifacts used to determine if a suspect computer was used in the composition or review of messages using either the Twitter® web platform or the TweetDeck® desktop application. Although geared primarily toward an audience of digital forensics investigators/analysts/examiners, it is also well suited for attorneys, paralegals, or other legal professionals who often deal with evidence emanating from social media platforms, such as Twitter®.

The web interface will be tested through a variety of browsers, to include Microsoft Internet Explorer® (versions 8 and 9), Mozilla Firefox® (version 14), Apple Safari® (version 5), and Google Chrome® (version 20). The TweetDeck software will be tested by downloading and installing it on a clean installation of Windows 7. Three dummy Twitter® accounts will also be created to aid in the testing process.

Founded in 2006, Twitter® is an online social media outlet that allows its users to post micro-blogs of up to 140 characters called "tweets." The rapid growth and acceptance of Twitter® by the public is evidenced by the fact that the company now has over 500 million users; and, according to the web information site Alexa, their most recent three-month tracking numbers show that Twitter® is the eighth most popular website in the world.[1] Its social significance can also be gauged by the enormous popularity of segments on late-night talk show television programs where celebrities appear on the show to read mean-spirited tweets about themselves.

Although there currently exist multiple third-party options from which a user can access and utilize a Twitter® account (i.e., HootSuite, Tweetings, Echofon, etc.), a recent article on TechCrunch.com cites statements made by the founder of Semiocoast, a French social media monitoring company, that "Twitter's® own access points, including TweetDeck, represent 75.4% of all public tweets."[2] This statistic was used to determine the most probable methods by which Twitter® artifacts would be generated, leading to the analysis performed for this presentation.

A prime example for the need of this type of analysis can be found in a 2011 case from the U.S. District Court for the District of Colorado, *Doe vs. Hofstetter*, in which the court found that the defendant created a fake Twitter® account, impersonated the plaintiff, and "communicated with third parties using the fake Twitter® account."[3] In this particular matter, knowing the types of artifacts left by the usage of Twitter® through either the web interface or through TweetDeck could have proven beneficial to those examiners investigating the defendant's computer. Additionally, the high-profile matter involving inappropriate tweets that may or may not have been sent from former Representative Anthony Weiner's Twitter® account highlights the need for reliable research to identify what, if any, artifacts are left behind on a computer by Twitter® usage.

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Twitter®, Social Media, Digital Forensics

B14 Cell Phone Images in Social Media

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After attending this presentation, attendees will understand how to differentiate images originating from different cell phones. Furthermore, attendees will be able to differentiate images when they have been uploaded to a social media website.

This presentation will impact the forensic science community by providing a tool for law enforcement to track the origin of cell phone images posted to social media websites including Facebook®, MySpace, and Twitter®. This presentation will seek to eliminate the mystery that has surrounded images uploaded to the Internet.

Social networking websites have become a group depository of personal photos for all to see. The public availability of these images provides evidence otherwise unavailable to law enforcement; however, this valuable investigative tool comes with its drawbacks.

Very little is known about images uploaded to social networking websites. Previous successful research has been done into the usefulness of artifacts left by social networking websites, but no known research has dealt with uploaded images.[1] Currently, images uploaded to social networking websites cannot be traced back to their origin. This

presentation will provide a tool to track the origin of cell phone images posted to social media websites.

Traditional eyewitnessing of crimes is being replaced by video and image recordings. Today, almost everyone owns a cell phone and crimes are being increasingly captured on cell phone cameras. Unlike the traditional camera, people always carry their cell phones, and they are becoming increasingly user friendly.

As society is technologically evolving, evidence of crimes is increasingly posted to social networking websites. Investigators must determine where cell phone photos that find their way onto social networking websites originated. Investigators need to be able to trace exactly which social networking website an image came from and the cell phone that captured the image, but the upload process makes it difficult for investigators to determine where there images originated.

Cell phone cameras create photos in a unique way, providing a method of identification. Social networking websites alter uploaded images to normalize and reduce file size, providing a distinctive signature. Identifiers from the unique method that cell phone cameras use to capture photos and the distinctive signature produced by social networking websites are the basis of this study.

A database of cell phone camera images has been compiled to determine the unique signatures. These images were then uploaded to social media websites including Facebook®, MySpace, and Twitter® to determine which unique signatures remained for identification. The alterations done by the social media website image upload process were then recorded and compared against other social networking websites.

The database of cell phone camera images will be compared with test images of unknown origin to determine the cellphone camera that captured the photo and the social networking website that it was uploaded to. The database of images uploaded to social networking websites will be compared to determine distinctive signs of each of the social networking websites uploading process.

The final results of this research will show that images uploaded to the Internet can be identified given a sufficient database of images to compare, and known identifiers of cell phone cameras and social networking website upload systems.

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Facebook®, Cell Phone, Social Media

B15 The Facebook® Faker: Case Study of an Online Romance Scam Turned Dangerously Physical

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The goal of this presentation is to provide an example of the use of Cyber-Physical Crime Assessment (CPCA) techniques to characterize a crime, crime scene, and suspect, thus informing the investigation. The presenters will describe the case and apply CPCA to explain its ramifications and how CPCA might be used as an investigative tool to support a digital forensic investigation.

This presentation will impact the forensic science community by suggesting that CPCA may be useful for predicting the point at which a cyber-stalker, cyber-bully, or cyber-harasser may resort to actions and violence in the physical world. The case study supports Jaishankar's space-transition theory in cyber criminology.[1] It represents one type of romance scam where the scammer uses social media to troll for and groom victims before attempting contact in the physical world. This case study is part of the presenters' on-going research into CPCA and its direct applicability to digital forensic investigations.

DK is a 49-year-old male who entraps women and men using Facebook®, arranges meetings with them, and escalates many of those meetings to sexual encounters often with (up to 79%) violent assault. Over his history, DK has engaged with at least 84 victims. Typically, he engaged the victims using a variety of subterfuges. Likewise, he often attempted to develop a real-world relationship at the point where he perceived that he was not achieving adequate control over his victims online.

DK is overweight, indigent (living in shelters when he does not have a victim with whom to live), and expert in the use of the Internet and social media, which he accesses either from a library or using a victim's computer and Internet connection. He is reported by one of his victims to "…sleep no more than four hours per night" spending much of the rest of his time online.

He represents himself on Facebook® as younger, a bodybuilder, extremely wealthy, an ex-FBI agent, and a former private sniper who was paid by the kill. None of these is true. He uses a variety of aliases and is suspected of both identity theft and child abuse. In interviews with his 84 victims, several have indicated that he raped them; however, most women with whom he has been involved did not report him because he was careful to engage initially in consensual sex and, where possible, cohabitate with his victims.

DK represents the Power Reassurance (PR) sub-type and spikes over into the power assertive (PA) sub-type when he can no longer satisfy his need for control of his victim.[2] The PR sub-type is characterized, among other things, by the actor living in a personal fantasy. The fantasy drives his actions. The PA sub-type is characterized by aggressive assertiveness.

In the case of DK, he lives in the fantasy that he is the person he represents himself to be (PR). Then he attempts to engage his victim in *his* fantasy and, when he no longer can control his victim, he exerts more power and his actions become PA. When he no longer can exert control through his online presence, he attempts to move the relationship into the real world, often with violent results.

Analysis of chat logs, a sting operation, video of interviews with DK in the media, his emails, and tracking of his numerous Facebook® presences has allowed the presenters to develop a very clear picture of DK's behavior. Additionally, the presenters have had access to computing devices (including laptops, tablets, and smart phones) that DK used extensively. Applying CPCA to the forensic analysis of these devices allowed the presenters to test the viability of considering the computing device as a crime scene that could be analyzed in much the same way that a crime scene investigator analyzes a physical crime scene.

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Facebook®, Crime Assessment, Cyber Crime

B16 Taking a Deeper Look Inside Microsoft's XBox® 360: The Acquisition and Investigation

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The goals of this presentation are to discuss: (1) data preservation and forensic collection from an XBox® 360 system; (2) forensic investigation of an XBox® 360 system; and, (3) differences between the XBox® 360 FATX file system and other computing systems, such as Windows™ and Macintosh-based file system structures and file formats.

This presentation will impact the forensic science community by providing basic tools and techniques used to forensically acquire information of evidentiary value on XBox® 360 gaming devices.

The Microsoft XBox® 360 with Kinect is the latest online gaming device on the market. The addition of the Kinect gives the XBox® 360 the ability to control games without using a controller, to record voices, and to snap digital images of the users. The XBox® 360 is recognized as

a popular entertainment and educational device, as well as a platform that can be used by online predators to victimize children or store evidence of criminal activity. Because the XBox® 360 can be used in criminal activity, it is vital that digital forensics examiners be able to recover items of evidentiary value. While some research has been conducted on forensic examination of the XBox® 360, no known studies have addressed the evidence left on the XBox® 360 with Kinect. This study examines the evidentiary artifacts left by users on the XBox® 360 with Kinect and the implications for digital forensics examiners.

The first step is being able to capture the image of the drive used by the XBox® 360 system. However, despite it being able to be imaged as any other hard drive would, the file system is unsupported by Windows and can only be read by a special program. This program, known as Xplorer 360, is the only known program on the market today that has been able to read, write, and retrieve information from a hard drive used by an XBox® 360 system. Using this program, examiners will be able to retrieve information from a user's game history, saved games, achievement lists, gamer information; even photos and video bites captured from the game.

Once the basics of acquisition have been covered, a basic overview of the XBox® FATX file system will be provided so participants will be able to familiarize themselves with the system and the differences between Windows or Mac OSX file systems as well as be able to find information kept in the memory of the system taken from the XBox® 360 Kinect that is or may be of evidentiary value. A basic overview of how to use the program Xplorer 360 to retrieve such information taken by the XBox® 360 Kinect will also be provided.

Due to increasing XBox® 360 with Kinect system sales, working with these gaming devices, being able to study how they are being used in criminal activity, and understanding the system so one can retrieve any information that may be of evidentiary value is essential as the future of this technology grows. Such knowledge would prove useful in any forensic investigator's toolbox in that, should the crime related to it ever arise; such information would be greatly beneficial to getting one step closer to solving the puzzle.

Digital Forensics, Xbox® 360, Investigation

B17 Connecting Digital and Physical Crime Scenes Using Cyber-Physical Crime Assessment

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After attending this presentation attendees will be introduced to a novel method of conducting an investigation where there are both digital and physical data to consider. Typically investigators seize computing devices (e.g., desktop computers, laptops, smart phones, tablets, etc.) and submit them to a digital forensics laboratory for analysis. Digital forensic examiners perform tests on the devices using digital forensic tools to extract typical data and through iterative communications with investigators search for individual pieces of data of possible interest in solving the crime.

This presentation will impact the forensic science community by demonstrating how Cyber-Physical Crime Assessment (CPCA) shows promise as a method for conducting a holistic investigation of a hybrid crime (a crime with both digital and physical elements). CPCA connects the physical and digital crime scenes together as a single crime scene, each with its unique characteristics, but intended to be viewed and analyzed as an integrated whole in much the same manner as a crime scene investigator would analyze the physical scene alone.

CPCA holds promise as a methodology for conducting a holistic investigation of a hybrid crime, defining hybrid as one with both digital and physical elements.

CPCA applies physical crime/crime scene assessment techniques developed over three decades and is based upon informal analysis of over 20,000 cases of violent crime. The presenters have ported these techniques into the digital world and refined them to include both digital and physical crime scenes where a single, hybrid crime has been committed.

This type of crime assessment consists of three aspects: description of the crime/crime scene using one or more of four sub-types, matching the sub-type(s) of suspects to the sub-type(s) of the crime, and analysis of the pre- and post-crime activities of key suspects.

Temporal analysis of pre- and post-crime activities, for example, is straightforward on a computing device due to the timelines that can be constructed using such things as file metadata and machine activity (e.g., When was the computer turned on or off? What cell phone activity can be matched to the pre- and post-crime activity? How does cell tower access compare with timelines of the physical event?) By considering the physical and digital aspects of the crime as a single crime scene with an integrated clue set, these connections become more obvious and useful in solving the crime, guiding digital forensic analysis, and supporting the solution for a conviction.

The other key area of consideration is the four sub-types. These subtypes—power assertive, power reassurance, anger retaliatory, and anger excitation—are described in more detail in Stephenson and Walter, and Keppel and Walter.[1,2] Experience in physical crime has demonstrated that all crimes fit into one or more of the sub-types. There usually is a primary sub-type and there may be secondary sub-types with lesser weightings in a complicated crime.

The crime scene reveals the sub-type(s) and there may be indications that the crime has started as one sub-type and ended as another, further differentiating the potential suspects. This "spiking over" from one sub-type to another is easily seen in the digital aspects of the hybrid crime scene due to the ability to analyze computer use patterns and documents such as emails, Internet pages, and social network accesses/postings.

Once the timelines of the crime are established, the important suspects may be characterized using the same sub-type analysis as applied to the crime/crime scene. This reduces the field of suspects to those who exhibit congruent characteristics to the crime. Those primary suspects are then analyzed based upon their pre- and post-crime activities, again searching for congruence with the physical event, and a smaller, more manageable sub-set of credible suspects is likely to emerge.

CPCA is applicable to hybrid investigations where the perpetrator is unknown but a field of suspects exists as well as investigations where the perpetrator is known and support for interview/interrogation and prosecution is needed. The presenters have applied CPCA in actual investigations and are currently analyzing empirical data for statistical correlation with theoretical constructs.

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Cyber Crime, Crime Assessment, Digital Forensics

B18 Computer Forensics Tool Catalog: Connecting Users With the Tools They Need

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After attending this presentation, attendees will be introduced to the Tool Catalog website, a website that provides users with an easily searchable catalog of forensics tools. The secondary goal is to provide a map of the computer forensics tool landscape, showing where there are gaps (i.e., functions for which there are no tools). The attendees of this presentation will become familiar with the Tool Catalog website and the benefits it offers the forensics community.

This presentation will impact the forensic science community by: (1) the forensics practitioner will have a better, more efficient way of connecting with the tools and technologies they require to do their work; and, (2) the law enforcement community will gain the ability to better identify and communicate the functionalities and features they need to the development community. This is facilitated by the map of forensics functions—the Tool Taxonomy.

There are many forensics tools available. These tools perform a wide variety of functions (e.g., imaging or memory reconstruction) and work on a wide variety of technologies (from PCs to Macs, to disposable phones, to iPhones). What is missing is an effective way of connecting practitioners to the tools that meet their specific requirements. The computer forensics tool lists currently available to the community have limitations in how they are populated, and in the level of detail of information they provide about tool capabilities. Maintaining a list is time consuming and lists easily become outdated. Also, while these lists generally connect practitioners to tools, they lack specificity. For instance, a typical tool list entry for a disk imaging tool might tell a practitioner that a tool supports USB, FireWire, and SCSI drives, but will not specifically address if this support is for the tool's evidence interfaces, target interfaces, or both. It will not uniformly list the specific capabilities of similar tools, such as for a disk imaging tool: the hash algorithms and image file types they support; supported acquisition methods (disk-to-disk or disk-to-file); whether it can encrypt data; if it supports block hashing, etc. Such specifics are important for connecting practitioners to the tools they require but are very time consuming to collect.

The website has three major sections. First, there is a description of forensic functionalities and technical parameters. This is the Tool Taxonomy. Currently, eight functionalities are defined, along with associated technical parameters and technical parameter values. These allow a tool's capabilities to be characterized. Example forensic functionalities are disk imaging, deleted file recovery, and mobile device acquisition and analysis. Example technical parameters for disk imaging include runtime environment, evidence interfaces, target interfaces, supported image file formats, hash algorithms, and acquisition methods. New functionalities will be added to the Tool Catalog based on the work of the Computer Forensics Tool Testing project.

Second is a search feature to find tools. Users can select their requirements in terms of functionality needed as well as specific technical parameter values. A list of tools that match the search criteria is returned. For example, a user might formulate a search for "all disk imaging tools that have support for acquisition to a networked file system, support the dd, E01, and AFF image file formats and support the SHA256 hash algorithm."

The third section of the Computer Forensics Tool Catalog site is a set of pages for vendors to input information about their tools and for public feedback. Unlike traditional tool lists, the Tool Catalog is not populated by a single entity or via crowd sourcing; the website is populated with tools by tool vendors. Tool submissions are reviewed at NIST prior to being posted to the website. Two advantages of this approach are that it takes the pressure off the single person or entity of keeping abreast of the latest tool developments and it leaves the task of populating the tool's specific capabilities to the party who knows them best, the vendor. Comments and feedback from both vendors and the forensics community are welcomed and are key for improving and keeping the Tool Catalog current.

Digital Forensics, Tool Taxonomy, Website

B19 Deleted File Recovery Tool Testing Results

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After attending the presentation, attendees will learn about issues revealed while testing meta-databased deleted file recovery computer forensic tools by the Computer Forensics Tool Testing (CFTT) project.

The presentation will impact the forensic science community by increasing awareness in the community of tool test strategies and the ability of tool testing to reveal anomalies in tool behavior. The presentation will aid the forensic practitioner in recognizing the limitations of meta-databased deleted file recovery tools.

The CFTT project develops methodologies for testing computer forensic tools. This presentation reports on tool behaviors observed while testing digital forensics tools against a set of file deletion scenarios.

A file system is used to store data for access by a computer. The data is normally stored within a tree-structured hierarchy of directories and files. When a file or directory is deleted from a file system, the associated *metadata* entry and the stored data are no longer directly accessible to the user and appear to be completely removed. However, in many file systems, e.g., FAT, neither the metadata associated with the file nor the actual content is completely removed. This creates a situation where there is *residual metadata* (metadata remaining after a delete has occurred) that is still accessible by direct access outside the usual operating system methods and can be used to reconstruct deleted files. Many forensic tools exploit the behavior exhibited by file systems of leaving metadata behind after a file is deleted to attempt to recover deleted files. Metadata-based deleted file recovery should not be confused with *file carving*, i.e., scanning unallocated memory for the file signatures present within a file itself to identify a deleted file. The scope of this presentation is limited to metadata-based deleted file recovery tools that use file system metadata from file system structures such as directories or i-nodes to identify recoverable deleted files.

The basic approach to creating a test image is as follows:

1. Create a file system on a secondary storage device.
2. Create some files.
3. Delete some of the created files.
4. Image the storage device.
5. Use the tool under test to attempt to recover the deleted files.

The test images used cover the most widely used file systems, including FAT16, FAT32, ExFAT, NTFS, ext2, ext3, and ext4. The HFS+ file system does not leave enough residual metadata behind after a file is deleted to make recovery practical.

Some of the observations discussed in this presentation include:

- The residual metadata varies with the file system. For example, file names may be completely or partially lost, pointers to file blocks may be overwritten.
- Only the first block of a deleted file is identified for FAT16 and FAT32 file systems. Some tools guess the location of the remainder of the deleted file; this strategy often leads to recovered files that are mixed from several original files.
- The tools sometimes include blocks from active files in a recovered file.
- The tools rarely include blocks that have never been allocated to the current file system, i.e., it is not likely that a block from a recovered file was not a part of some file.
- Some tools attempt to identify overwritten files. The tools often identify (incorrectly) intact files as overwritten.
- Support for ext3 and ext4 is often lacking.
- Sometimes ExFAT is not supported.
- Interpretation of MAC times must be done carefully. Time zone information and actual semantics of the times can vary across file systems and tools.

The test images and image layout documentation are available at the CFReDS project web site http://www.cfreds.nist.gov/dfr-test-images.html. Test reports on specific tools are available for the National Institute of Justice web site http://www.nij.gov/topics/forensics/evidence/digital/standards/cftt.htm

File Recovery, Software Tool, Digital Forensics

B20 A Survey of Data Deletion Applications for OS X Systems

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After attending the presentation, attendees will have a general understanding of the types of data deletion software currently available

for OS X systems, the basic functions of each program, and examples of artifacts left behind by each program upon installation and usage.

This presentation will impact the forensic science community by providing useful information for digital forensics examiners to assist in identification of data destruction activity on suspect computers running the OS X operating system. Although geared primarily toward an audience of digital forensics investigators/analysts/examiners, it is also well suited for attorneys, paralegals, or other legal professionals who often deal with the ramifications of the answers provided to the question posed at the outset.

During the course of an investigation, digital forensics investigators are often presented with this relatively simple question by prosecutors, defense attorneys, or supervisors—"Did the person delete anything from their computer?" The reality is, while the question in and of itself is seemingly simple, depending on the type of operating system in play, attempting to determine the answer to that question can be devilishly difficult.

Over the years, the Digital Forensics (DF) community as a whole has developed multiple tools and techniques to assist in answering that question. Drawing on one of the core tenets of criminological theory, Locard's Exchange Principle, one of those techniques is to identify what artifacts are left behind by the installation and subsequent running of data destruction software. Although considerable resources have been spent by the DF community to identify those artifacts—both from the software itself as well as from the operating system—the vast amount of those resources were spent primarily on computers running some version of the Windows operating system. Th goal is that this presentation helps to change that approach.

With the growing popularity of Apple's Macintosh family of computers, more resources will need to be expended to increase the community's collective knowledge of the same types of artifacts that have already been identified on Windows-based systems. This further affects the DF community because, while computers running Windows operating systems still have a virtual stranglehold on the corporate market, a January 2012 article in the *Wall Street Journal* reported that General Electric has a pilot program that allows its employees to choose Apple desktops or laptops running OS X instead of a PC running Windows. According to a *Wall Street Journal* article, around 1,000 employees are currently using an Apple computer (which represents less than 1% of the overall users at G.E.); however, more employees are taking advantage of the program as it becomes more widely known.[1]

A prime example of the disparity between the emphases placed on research of data deletion artifacts between Windows and OS X platforms can be found in one of the well-respected digital forensics books on the market today,–*Handbook of Digital Forensics and Investigation*, edited by Eoghan Casey. In Chapter 5 of that book, titled "Windows Forensic Analysis," six pages are devoted to the topic "Deletion and Destruction of Data."[2] In comparison, Chapter 7 of that book, titled "Macintosh Forensic Analysis," contains no such section; however, references to data deletion can be found scattered throughout the chapter.[3]

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OS X, Macintosh, Data Deletion

B21 Bulk Data Analysis With Optimistic Decompression and Sector Hashing

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After attending this presentation, attendees will understand the difference between file-based and bulk-data approaches to digital forensics, and how bulk data approaches can be significantly empowered through the use of optimistic decompression and sector hashing.

This presentation will impact the forensic science community by providing results from controlled experiments in an area with little previous research, adding to work being carried out in digital forensics by broadening the understanding of the presence of compressed data, especially fragmented compressed data, in unallocated areas of file systems, and the availability of new techniques to decompress such data without reference to file system metadata or file type. This presentation will also build upon previous research in sector hashing (also known as piecewise hashing and with hash-based carving and show how sector hashing can be empowered through the use of a high-performance custom-built database.[1-5]

Bulk data analysis is a new digital forensics technique that eschews file extraction, and instead focuses on the processing of bulk data read directly from the target media. Unlike file-based approaches, bulk data analysis is particularly well suited to triage, as it can be parallelized and applied to random sampling.

In the first experiment, a corpus of roughly 2,000 hard drives purchased on the secondary market was analyzed for forensically important information that could only be recovered through the use of optimistic decompression of sectors that were not contained within allocated or recoverable deleted files.[6] Optimistic decompression means that all decompression algorithms are applied to all sectors with the hope that some compressed data may be identified and decompressed. This experiment employed the use of the bulk-extractor, fiwalk and identify-filenames.py tools.[7,8] This study found a significant number of email addresses, URLs, account numbers, and other kinds of information that could only be recovered through the use of optimistic decompression. This presentation show how additional processing can be used to determine which recovered features are attributable to user-generated content, and which are residual data from software distributions.

In the second experiment, three corpora (GOVDOCS1, OpenMalware 2012, and NSRL) were hashed on sector boundaries and evaluated for the presence of distinct sectors—that is, sectors which are not present in any of the sectors in the corpus.[6,9-11] Many such distinct sectors are present and how they can be used to identify the presence of either intact files or to attribute residual data to specific files of interest will be shown.

In conclusion, these two studies provide evidence that bulk data processing can be productively used by digital forensics examiners for both triage and for the extraction of case-relevant details. Examiners can use the tools presented in this presentation today. This presentation will also provide sufficient information so these techniques can be embedded into other tools.

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Digital Forensics, Optimistic Decompression, Sector Hashing

B22 The Impact of Child Pornography Analysis on Military Legal Proceedings

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After attending this presentation, attendees will have a general understanding of the concerns associated with the digital evidence child pornography investigation process and gain an understanding of how results are currently being used by the military judicial system.

This presentation will impact the forensic science community by providing an understanding of the effect of child pornography on the outcome of judicial proceedings, discussing how advancements in technology and the current examination processes affect the digital evidence field in child pornography cases at the USACIL.

Recently, digital evidence examiners have experienced an increase of submitted evidence due to both better agent identification of digital evidence as well as rapid advancements in technology. Storage devices will soon have the capability to hold over 60 terabytes of data. There does not exist sufficient time or manpower to effectively and efficiently keep up with today's constantly developing technology. Currently, during an investigation, digital evidence examiners at the United States Army Criminal Investigation Laboratory (USACIL) must duplicate and examine all submitted evidence, which is not only time consuming but requires a large amount of digital evidence storage capacity for both examination processing and subsequent storage of the case file. Focusing specifically on child pornography, an issue prevalent in military investigations, some form of limitation must be created to maintain current case output with the increasing amount of data required to be examined.

To facilitate this study, 66 U.S. Army case files dating from 2009 to 2011 were collected, all of which involved child pornography. Information such as the date the exam began and finished, number of digital evidence exhibits submitted, number of exhibits with child pornography, number of exhibits with information of possible interest to the investigation, as well as the amount of child pornography found on each specific device was recorded. Once all the applicable information was attained, the results showed that a large portion of exhibits submitted did not contain child pornography. The study continued by determining which cases included submitted pictures/videos that were identified by the digital examiners and sent to the National Center for Missing and Exploited Children (NCMEC). The number of pictures/videos identified by digital examiners and confirmed by NCMEC as child pornography was recorded to determine the percentage of cases where examiners successfully identified previously known and identified child victims. All of this information was collected in order to characterize which cases had confirmed child pornography and if that had an effect on the overall judicial outcome of a case.

To determine the effect of child pornography on the legal outcome of a case required each case's Commander's Report of Disciplinary or Administrative Action (DA Form 4833) and a review of case documentation within the final forensic reports. Items that were collected for correlation analysis included: the number of pictures/videos identified, numbers of items submitted for analysis, the types of items submitted, and the results of the disciplinary action. It was expected that the results would show a relationship between number of items submitted, the number of photos/videos identified, and the outcome of the judicial process. It should be noted that in 2012, the Uniform Code of Military Justice (UCMJ) implemented a revision to the code concerning child pornography, which affects how individuals are charged and sentenced pertaining to these cases. It is expected that with these modifications, there will be a more direct relationship between the number of photos/videos and the severity of punishment.

*The opinions or assertions contained herein are 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.

Child Pornography, Digital Evidence, Judicial Impact

B23 Optimal Distance Determination for Reference Recordings Using Reference Subtraction Filtering in Noisy Environments

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After attending this presentation, attendees will understand the importance of reference subtraction filters using reference recordings made simultaneously with targeted conversation recordings in noisy environments, such as restaurants, coffee shops, or bars, to determine optimal distance from the recording reference to the target to optimize the filter's response.

This presentation will impact the forensic science community by serving as a best practice or standard when making a covert recording of a subject in noisy environments.

Reference subtraction filters work by performing a continuous and intelligent subtraction of one audio signal from another. Normally, with a forensic recording containing speech masked by loud audio, one would require a reference recording containing just the audio that needs to be removed. The audio track containing the music or other audio to be removed is called the "Reference Track." This type of filtering has had great results in a real-time reference capturing environment. It is done by placing two microphones in the area of the target conversation. One is placed near the target conversation, and the other near the source of the background noise or audio source such as a TV, stereo system, jukebox, or a live band. Then, using a reference subtraction filter that can operate in a live situation, the reference noise signal can be subtracted from the source to produce a more intelligible target conversation. These types of two-microphone, live recordings take a lot of coordination and are usually done when a hotel room is used for the targeted conversation. There is a need to use reference subtraction type filtering in a post-processing environment.

To make reference subtraction filtering effective in a noisy multi-signal environment, two separate recordings need to be made simultaneously. Making a reference recording at some distance from a targeted recording in a noisy background environment will aid in decreasing the noisy background. The key variable is determining the optimal distance that a reference recording should be made from the

targeted recording in the same noisy environment, and then use a reference subtraction filter to make the targeted conversation more intelligible.

A series of reference and source recordings will be made in different noisy environments (e.g., restaurants, coffee shops, or bars). The source recording will be made to simulate a covert recording of a suspect while in the noisy environment. The reference recording will be made simultaneously to the target recording in the same environment, but at different distances, in an attempt to eliminate the noisy environment from the target. The filtering will be done with several popular reference subtraction filters made by different audio forensic processing systems.

Real-time and post-reference subtraction filters are widely used and have been proven effective in reference subtraction of a single sound source broadcast television show or music recording. What about reference subtraction in multiple sound source situations such as restaurants, bars, or coffee shops? In many covert law enforcement targeted conversation recordings taking place in these noisy environments, tests will be done to determine the optimal distance to make a reference recording that would subtract the noisy background most effectively. Test recordings will be produced in several different noisy environments using the same type of recorders and microphones with the only variable being the distance from the source recording. The improvement in intelligibility will be reviewed aurally, spectrographically, and with signal-to-noise ratio measurements.

Audio, Enhancement, Multimedia Forensics

B24 Audio Authentication: A Comparative Analysis of Same-Room Recordings vs. Speakerphone Recordings

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After attending this presentation, attendees will understand a methodology for examining recorded voices, with and without telephone transmission effects, to determine if conversations were recorded under conditions in which they were allegedly created.

This presentation will impact the forensic community by demonstrating a process to determine the recording conditions of an audio signal or dialogue. It will also demonstrate some ways to manipulate one set of recordings to attain similar properties of another. Analyzed material will include audio signals and speech recorded from the same room and between telephonic devices, via analog and digital audio recorders.

This research was inspired by an actual case that pertained to a conversation recorded on a microcassette. The recording was allegedly made by the victim using the recorder held to the earpiece of a cellular phone during a conversation during which he was threatened. Investigators reviewed the microcassette tape and questioned the authenticity of the recording as it sounded rehearsed. The recording was submitted for analysis to determine if the recording was produced as alleged, or if both parties were actually in the same room and possibly reading from a script.

This research will also explore techniques used to alter the characteristics of a same-room conversation to appear consistent with a two-location telephonic conversation. The ability to differentiate between recorded conversations occurring in the same room and those transmitted between telephonic devices can be an essential element of an audio authentication examination. Determinations concerning the authenticity of questioned recordings have the potential to change the dynamic of an entire case or investigation. Since a purely aural designation of tonal quality can be a subjective assessment and may vary from examiner to examiner, it is significant to point out the statistical, objective approach to measurement and characterization of the recordings used for this presentation. This approach provides a more reliable and statistical evaluation process is desired.

For this research project, several test recordings were made over different types of cellular and land-based phones, using a variety of recorders. To account for and quantify the properties of chosen recorded signals, these test recordings were analyzed using forensic audio tools, signal processors, and forensic phonetic software. The recordings were characterized by their reported correlation coefficients and mean quadratic difference values, as well as the average frequencies and standard deviations of each voice formant in each recording scenario. The comparison methods allowed for an established baseline to identify the differences between same-room and speaker phone recordings, which in turn provided a clear goal for the next element of the project.

Utilizing similar audio tools and techniques, researchers attempted to duplicate the qualities of a speaker phone recording with a manipulated same-room recording. After manipulation, the newly generated recordings were compared against same-room and speaker phone recordings of the same voice, using the same means as outlined above. A final determination was then made in regard to the effectiveness of the manipulations, and whether they rendered the forged recordings more or less similar to either scenario. This presentation will highlight the preparation, execution, and results of this study.

*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.

Audio Comparison, Audio Voice Analysis, Audio Authentication

B25 Electric Network Frequency (ENF) Database Validation for Digital Media Authenticity

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After attending this presentation, attendees will better understand Electric Network Frequency (ENF) analysis for forensic media authentication and criteria necessary for creating, maintaining, and validating a database of ENF fluctuations.

This presentation will impact the forensic science community by discussing inter- and intra-variability tests conducted at the National Center for Media Forensics (NCMF) to analyze random fluctuations of ENF and the hypothesis that ENF databases would not contain repetitive sets of data.

In order to determine the time/date of a recording made where ENF is present, a time-synchronous database of ENF variations must be available and have been recorded during the time in question. At the NCMF, the ENF database configuration records non-stop ENF variations of the U.S. Western grid with an 8kHz sampling frequency and 16bit, mono, uncompressed files. Recording of ENF is redundantly made on two completely isolated computers. The NCMF is in partnership with the Target Forensic Services Lab (TFSL) in order to create a network of ENF databases currently covering the western and eastern U.S. power grids. Sites include: NCMF Denver, CO, TFSL Las Vegas, NV, and TFSL Minneapolis, MN. This network is under constant evaluation and testing and will soon be expanded to cover the Texas grid. Bi-annual validation of ENF information between the NCMF/TFSL ENF databases is scheduled which will help ensure database integrity and meet forensic best practices. Several databases have been reported in continental Europe and the UK.

After having analyzed ENF variations in Western U.S. grid over a period of one year, and continental Europe for more than nine years, it has become clear that there is no precisely repetitive pattern. The fluctuations over time around 50Hz or 60Hz are purely random. Exceptions can be considered where some events occur during the morning and in the evening that are generated especially by maintenance operations or network components switching on or off.

Even so, their shapes and values cannot be predicted. In order to maximize confidence in results yielded from ENF examinations, the method proposed by the authors relies on redundancy data at each stage of the examination: two ENF databases, minimum of two techniques for ENF extraction (Fast Fourier Transform, Zero Crossings, and/or Spectrogram), and two methods of automated ENF comparison (correlation coefficient mean quadratic difference).

This presentation will discuss inter and intra -variability tests conducted at the NCMF to analyze the random fluctuations of ENF and the hypothesis that an ENF database would not contain repetitive sets of data. Analysis was conducted using the Denver and Las Vegas databases, and both correlation coefficient and mean quadratic error methods. This consisted of comparing sets of one- to ten -minute segments against all other segments within that same period and evaluating the maximum correlation coefficient (CC) and minimum mean quadratic difference (MQD) for the corresponding and different time frames. Results indicate two things: (1) that each ten-minute segment is much more correlated to itself than any other ten-minute segment within the recorded 18-month databases; and, (2) that MQD provides better discrimination power than CC.

Labs coordinating the acquisition and archival of ENF data must be aware of potential dangers and take precautionary measures to ensure data maintains integrity and accuracy. This must extend to the procedures employed in forensic examinations. This presentation will provide a proposed format for validating database material as well as a list of known potential errors and suggested corrective actions. These are mandatory in order to meet the strict demands for accuracy and consistency of forensic materials to be used in labs, along with the development of and adherence to best practices and guidelines.

ENF, Audio Forensics, Database Validation

B26 An Approach to Conducting Automated Speaker Recognition

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The goal of this presentation is to instruct participants regarding training and testing preparations prior to performing automated speaker recognition forensic examinations.

This presentation will impact the forensic science community by giving participants knowledge and insight into the training, testing, and procedures used at the FBI audio laboratory involving Automated Speaker Recognition (ASR) forensic examinations.

The automated speaker recognition technology has made significant progress in performance and accuracy in the past decade due to the rigorous and worldwide research effort. Although the performance and accuracy continues to improve, there also remain many unresolved challenges. An approach will be described to resolve such challenges in performing forensic voice comparison examinations by the use of an ASR system currently used at the Federal Bureau of Investigation for investigative use. The approach is described as an automated speaker recognition system with a human-in-the-loop which has channel-independent and language-independent capabilities. The ASR system is built on MIT Lincoln Laboratory's state-of-the-art algorithms i-Vector, Joint Factor Analysis, and Inner Product Discriminant Functions (IPDF) operated by a trained examiner. Some recent improvements have been made in the operating procedures, log Likelihood Ratio (LLR), score interpretations, test and validation methods for LLR score calibrations, examiner training curriculum, and an innovative automated human examiner training module. The final decision-making process uses five levels: "match," "probable match," "inconclusive," "probable no-match," and "no-match" based on the fused results of the ASR system and the human examiner.

The ASR examiner training consists of classroom sessions taught in-house and lectures provided by leading academic organizations, and self-paced peer-review sessions to establish whether trainees have mastered the skills and knowledge required to conduct ASR examinations. The entire training process was monitored for each trainee by a designated mentor and is documented using an ASR training curriculum. A six-month-long classroom instruction was conducted for examiner-trainees by a senior voice comparison examiner, consisting of basic audio signal processing, basic science of speech production, speech perception, phonetics, and statistics and probability theories. A specialized self-paced training program called the Forensic Training Module (FTM) is administered to each examiner-trainee to perform and successfully pass a set of more than 100 blind voice comparison tests under cross-language (the training speaker speaks language 1, while the test speaker speaks language 2), cross-channel (the training speaker speaks via telephone, while the test speaker speaks via microphone) by listening only. All voice samples in the databases used for the FTM training are truth marked, thereby providing the trainees immediate feedback for optimum learning purposes. It was determined that through the ASR development process, there are specific acoustic conditions which are encountered when conducting forensic voice comparison examinations. These conditions and the characteristics of each will be discussed.

The testing and validation of the ASR system was a culmination of years of development of automated tools, including Air Force Research Laboratory's RAPT-R system and Massachusetts Institute of Technology Lincoln Laboratory's Vocalinc system. A Test and Validation Plan was prepared and approved for the voice comparison analysis of thousands of known voices. The ground truth of the databases allowed LLR-score thresholds to be calibrated for five levels of decisions: match, probable match, inconclusive, probable no match, and no match for each of 14 separate acoustic recording conditions. Consequently, examiners use the ASR algorithms to calculate automated scores for questioned voice recordings. Research and testing to improve the ASR process continues, addressing other factors impacting the ASR performance including speech duration, signal-to-noise ratio, and the degree of reverberation in the environment.

The current policy and limitations of the use of ASR as a courtroom tool as well as some *Daubert* criteria challenges will be presented. Finally, the progress status of a new scientific working group for voice (tentatively called SWG-V) will be discussed; formulation of this group is under way and due to the joint efforts of the National Institute of Standards and Technology and the Federal Bureau of Investigation collaborating with scientific representatives from local, state, federal laboratories, academia, industry, and legal community.

ASR, Speaker ID, Voice Comparison

B27 Framework for Authenticity Analysis of Digital Audio

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After attending this presentation, attendees will better understand the authentication process for digital audio, using ways in which various analyses can be combined into a robust framework.

This presentation will impact the forensic science community by providing a logical and scientifically sound process for digital audio authentication.

Digital audio authentication is a complex process of establishing the provenance of a questioned recording to determine whether it is consistent with an original one or if there is evidence of tampering. It also must follow the basic principles of forensic science that include five stages: (1) occurrence of the crime (or acoustical event); (2) seizing of evidence (no material contamination of media support and no digital contamination of the data); (3) analysis; (4) interpretation; and, (5) presentation. This paper presents the organization of several

techniques in a logical manner for the authentication of digital audio. Special attention has been given to interpreting results from individual analyses and incorporating them into a holistic view of a recording's authenticity where a finding can be corroborated against the results of other analyses. Only in this way can an examiner present a conclusion with confidence and assurance that all possible hypotheses have been exhausted in the execution of this important endeavor. The proposed digital audio authentication framework involves accurate, repeatable, reliable, unbiased, and scientific analyses that come from peer-reviewed publications in order to meet the *Daubert* standard, the NAS Report recommendations, and/or appropriate criteria of international legal systems. A forensic lab should also not accept tasks that their facilities, methods, software, databases, equipment, or specialist's background, training, and experience are not equipped to perform.

The task of digital audio authenticity can be separated into two main categories: container analysis and content analysis. The container relates to the file structure, metadata (either stored in the file itself or generated by a software or operating system), etc. Renaming of the container may not necessarily affect the integrity of the contents, but may alter and/or damage the media support or wrapper. This would raise doubts about authenticity that require explanation and may make some types of analyses inconclusive. Content analysis involves checking for traces of previous signal processing or editing using various methods such as: critical listening, waveform analysis, spectrogram, signal's power, dc offset, long-term average spectrum, sorted spectrum, differentiated sorted spectrum, compression level analysis, electric network frequency, butt-splice detection, analog transfer of a digital recording, reverberates analysis, MP3 edits, phase of a mono signal, and phase of a stereo recording. It should be noted that mono signal phase has certain limitations due to clicks, pops, clippings, and other signals from the environment. Also, in many cases, such as phone-intercepted recordings, reverberates, and MP3 edit analysis have limited or even no application. It is important for an examiner to show how they arrived at their conclusions and present them in a way that neither overstates nor understates the scientific certainty. Not every case will employ every type of analysis because, for various reasons, some may not be applicable; however, as many analyses as possible should be used in order to corroborate results. While the proposed table cannot account for every possible authentication technique or alteration technique known to exist, it should be useful in forming a reliable, ultimate conclusion regarding authenticity. The types of individual analyses that an examiner performs could be modified, expanded, or substituted as the tools and accuracy of the science improve over time.

This presentation will demonstrate the proposed framework in which an examiner would start at the global level and continue to the local level based on the findings and needs of the particular case. The appropriate philosophy regarding media authenticity will be discussed as well, for example when it is not scientifically possible to say that a digital audio file is absolutely authentic, or to prove that it is a certain clone/duplicate or copy of an authentic file. Therefore, language implying 100% certainty should be avoided unless speaking about known alterations or deletions. Whenever it is necessary, the results should be presented separately for container and content analyses, in a clear and unbiased way.

Forensic Audio, Audio Authentication, Methodology

B28 Unintended Consequences: Digital Forensics Literacy and the Legal System

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After attending this presentation, attendees will be able to achieve a retrospective of where the legal system stands with regard to digital evidence literacy, learn the potential consequences to the legal system if changes aren't initiated, and gain a description of one university's way forward.

This presentation will impact the forensic science community by voicing concerns over the alarming gap in the legal community's understanding of digital evidence. While the rule of law makes society a dependable environment in which to prosper and flourish, the lack of a predictable legal infrastructure has dire consequences.

Trust binds a society together. The rule of law makes society a dependable environment in which to prosper and flourish. The lack of a predictable legal infrastructure has dire consequences.[1] In this context, one must recognize that there is an alarming gap in the legal community's understanding of digital evidence, the technology community's understanding of how the legal system works, and how both can work constructively together.

In this presentation, one case is examined from a small community where the consequences of an inappropriate court ruling and a potential miscarriage of justice were avoided more as a matter of serendipity than of insight. Case specifics were examined, reminiscent of Amero,[2] and conclusions drawn about what this means generally to the state of digital evidence and its use in the justice system. Additionally, the evolution was traced of the general effectiveness of digital forensics evidence presentations and rulings, then extrapolate going forward as to where the evolution of technology may lead.[3-8]

Societal understanding, judgment, and decisions will always lag the development of technology; however, the consequences to the stability of the legal system as it slowly adapts to the changing nature of digital evidence and all that this implies, is staggering. Allowing the current state of digital evidence literacy to continue will likely include: decreasing trust in the predictability of legal decisions affecting the e-economy and, thus, the e-economy itself, and a general impedance of the progress of the Information Age—as online business and communications may increasingly no longer be viable or sustainable. It is incumbent upon those informed members of the technical community who are watching this potential train wreck evolve, to engage in dialogue with those communities that are impacted by the innovations but need help in digesting them and using them. Likewise, the technical community needs help in better understanding the practical ways the justice system—its laws, procedures, and decision process—work so that going forward, more relevant and effective innovations can be produced. Further, the legal system needs help in evaluating the validity and weight of evidence and other information in developing laws that affect judicial decisions.

One initiative being launched at a university law school in conjunction with an information school to deal with this problem will be presented. The goal of this study is that this example will ignite discussion not only about this effort but about what other efforts should be taken to improve the legal community's understanding of digital forensic evidence and the technical community's understanding of how the legal system works. Eventually, society does better understand technical innovation and adapts and evolves. In the meantime, inequities and even tragedies inevitably occur. This presentation will encourage dialogue about what this community can do to apply what has been most effective in understanding and using other forms of scientific evidence.

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Digital Evidence, Literacy, Legal System

B29 The Application of Virtual Machine Introspection to Digital Forensics

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After attending this presentation, attendees will understand the general concepts associated with Virtual Machine Introspection (VMI) and how VMI can be applied to recover data of interest to a digital forensics investigator. While virtualization is increasingly common in many environments, investigations tend to still be conducted using traditional approaches (e.g., static analysis of media or "traditional" live forensics using tools executed within the Virtual Machine (VM)).

This presentation will impact the forensic science community by describing and demonstrating new capabilities that can be applied to investigations in virtualized environments.

As virtualization becomes increasingly common, it is important to explore the extent to which existing techniques can be applied to virtualized environments, and what new forensic techniques may be applicable. VMI is a process by which the virtualization layer (e.g., a hypervisor) can unobtrusively inspect the complete state of a running virtual machine. VMI is particularly applicable to live digital forensics, as it allows the state of a running VM to be examined without requiring the investigator to install (or even run) software or tools on the VM under investigation, nor does it require that the investigator possess valid user credentials for the VM. Using this technique, forensically valuable data such as process lists, network connections, user activity, and memory contents can all be transparently extracted from the VM.

Recent work for this study (with funding from the DARPA Cyber Fast Track program) in the area of VMI has extended these capabilities to include the ability to gain direct access to cryptographically protected content on the VM. In particular, a digital forensic investigator can use this newly developed VMI tool set to examine a VM and recover cryptographic keys, monitor-encrypted (e.g., SSL and SSH) communication channels, and access encrypted files and storage volumes. Much of this content is either highly volatile or inaccessible without the cryptographic keys and, as such, would be unlikely to be accessible using more traditional digital forensics techniques.

This presentation will describe the applicability of VMI to digital forensics in general, and demonstrate its use to recover system data (e.g., process and network state information) and cryptographically protected content.

Virtualization, VM Introspection, Cryptography

B30 Messaging Application Analysis for Android and iOS Platforms

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After attending this presentation, attendees will have a better understanding of how to access information relevant to mobile device application usage and what remains in a smartphone's memory.

This presentation will impact the forensic science community by providing a straightforward explanation of what information can be found in the presence of specific applications on the two most common smartphone operating systems, Android™ and iOS™. This could help analysts efficiently process a smartphone found during an investigation by having knowledge of the type of information each application contains.

Mobile devices are a commonly encountered piece of evidence in today's investigations. The majority of the operating systems found on smartphones include versions of Android™ and iOS™. Both of these operating systems are enhanced by a multitude of third-party applications that can perform an almost infinite number of actions such as stock monitoring, banking, gaming, shopping, messaging, and photo enhancement.

This research, however, focused mainly on applications that had messaging capabilities which come in many different forms. Some applications are able to only send text and photo messages, referred to as traditional messaging; others are strictly Push-To-Talk (PTT) and operate similarly to walkie-talkies and only send audio messages. Another type of application combines both traditional and PTT messaging, referred to as multi-functional. A final type of application is one primarily for gaming, but allows players to send messages back and forth. These applications are attractive because they can be used strictly through Wi-Fi, which means they can be used on more devices and not require cellular service.

Using the number of ratings and number of downloads from Google Play™, the Android™ application market, seven applications present in both Apple's™ App Store™ and Google Play™ were chosen. Each one fell into one of the four types of messaging applications. For traditional messaging, WhatsApp Messenger™ and Facebook Messenger™ were chosen. For PTT messaging, Zello Walkie-Talkie™ was chosen. For multi-functional messaging, KakaoTalk Messenger™ and Voxer™ were chosen. For gaming applications, Words with Friends™ and Draw Something™ were chosen.

The applications were loaded onto an iPhone 4™ (iOS™ platform) and a HTC EVO 3D™ (Android™ platform) and used to test out the different capabilities of each application. After imaging the phones, the images were searched for artifacts about content and user information. The types of information searched were: text, audio and photo messages, sender/recipient information, location information, and timestamp information and were present in various forms. Text and photo messages were completely accessible except in Draw Something™. When enabled, all applications with location functions kept accurate tracking records and attached latitude and longitude coordinates to each message. Most applications also kept accurate timestamps. Audio messages were mostly unrecoverable, with Zello™ being the only application that could potentially have kept the original messages on the phone (denoted by a Speex file header). The greatest variability was in the user information kept. Not only did each application retain different information, but it also varied between platforms. The platform that generally kept more information was Android™; the Facebook Messenger™ app for Android™ recorded more information than seemed applicable to the scope of the application storing the phone number and e-mail address for each of the contacts.

Despite some limitations in the methods used, most of the information available from analysis of the messaging applications could be helpful in an investigation. Future research on mobile device applications would expand beyond just messaging applications to other types commonly found on seized phones. Another area of potential research would be to find a method that can be used to play the audio files from Zello™.

Smartphone, Messaging, Applications

B31 Mobile Device Tool Testing

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After attending the presentation, attendees will be aware of the importance of tool testing and gain an understanding of the mobile device tool testing process conducted within the Computer Forensics Tool Testing (CFTT) project.

The presentation will impact the forensic science community by increasing awareness of the role of tool testing in informing the forensic community of tool capabilities and limitations. Test reports provide a foundation for toolmakers to improve tools, help users to make informed choices, and provide interested parties with an overview of any anomalies found. The presentation will provide an overview of the motivation behind testing mobile device forensic tools and the challenges faced by toolmakers and forensic examiners.

The CFTT project has spent several years researching and testing forensic tools capable of acquiring data from the internal memory of mobile devices and Subscriber Identity Modules (SIMs). This presentation discusses all aspects of the testing process that are critical for producing a test report.

The development of mobile device forensic tools and acquisition techniques continues to grow within the field of digital forensics. Mobile subscribers far outnumber personal computer owners and studies have shown an increase of mobile device personal data storage compared to personal computers. Higher-end mobile devices present users with advanced features and capabilities similar to those of a personal computer. Mobile devices provide users with the ability to maintain contact information, upcoming appointments, day-to-day activities, important news events, and provide the ability to correspond with friends and family via telephony, text message, email, chat, and social networking sites. Over time, mobile devices can accumulate a sizeable amount of information about their owners. Data acquired from these devices may be useful in criminal cases or civil disputes.

As mobile device usage and sophistication continue to grow, so does the need for tool validation. For acquired information to be admissible in a court of law, verification of a tool's behavior and strict forensic acquisition methods are paramount. Potentially, one piece of data acquired from a mobile device may play a critical role in shedding light on an incident or, possibly, criminal activity. The need for rigorous testing conducted on a combination of forensic tools and specific families of mobile devices is critical for providing law enforcement and forensic examiners informative test results yielding known expectations of a tool's behavior, capabilities, and limitations. Over the past three years, the CFTT project at the National Institute of Standards and Technology (NIST) has tested numerous mobile device forensic tools capable of acquiring data from mobile devices operating over Global System for Mobile (GSM) communications and Code Division Multiple Access (CDMA) networks.

The presentation covers information on the motivation behind testing mobile device forensic tools, specification and test plan development, creation of a known data set, mobile device data population, and tool testing.

Mobile Forensics, Digital, Testing

B32 Using Visualization of Digital Forensic Datasets to Develop Fraud Detection Algorithms

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After attending the presentation, participants will have an understanding of abductive reasoning through visualization and an appreciation of the potential to leverage human visual processing with algorithmic development to detect and deter anomalous activities.

This presentation will impact the forensic science community by adding valuable tools and approaches to detecting anomalies in large data sets through visualization and the potential for using this information proactively to detect and prevent crime.

Mitigating card-present fraud through digital forensic analysis can be easy when the behaviors associated with the actions have been identified. The situation is much more challenging when one is not sure for what one is looking for. The human mind has a powerful ability to identify visual anomalies in large datasets. As part of a defense-in-depth strategy, this can contribute to the evolution and refinement of rule sets that facilitate the detection of the crime prior to the cash-out phase of the illegal operation. This presentation investigates the application of visualization, combined with human abductive reasoning, to the problem of identifying some behavioral characteristics of card-present fraud. It then demonstrates the behavioral characteristics in a digital forensics context, and demonstrates how this knowledge can be used to guide the evolution of analytical tools to help protect our digital assets.

The digital footprint left by individuals as they interact with technology throughout the day is astounding. As the use of Automated Teller Machine (ATM), credit, and debit cards becomes increasingly ubiquitous, the associated vulnerabilities make them more and more prevalent targets for cybercriminals. After a crime has been committed and identified, it may be possible to encode the observed information to develop an algorithmic approach to solving the problem. Since an algorithm is a step-by-step method to solve a problem or reach a goal state, clearly the process for solving the problem must be well understood. This works well when the problem is one that has been encountered, studied, and analyzed. What happens when new situations occur that are not expected? In this case, humans are able to reason abductively, and also excel at visual pattern recognition. This provides them with the ability to identify issues that are new.

Visualization is an excellent approach to facilitate these efforts as it allows the human analyst to see and evaluate datasets even when they do not know for what they are looking. Harnessing the power of visualization can provide a valuable tool in the arsenal to detect ATM usage anomalies including skimmed (counterfeited) card fraud. This presentation demonstrates how visualization techniques can be applied to couple the computational power of today's computers with the ability of the human mind to process images and reason through uncertainty. The example provided starts with abductive reasoning and is then analyzed and discussed reflexively. The intent is to demonstrate the value of visualization, in this case using parallel coordinates as a visualization tool, to identify anomalous patterns in data. The analysis of the findings can lead to the tuning of algorithmic tools to detect, and potentially prevent, future attacks using the same *modus operandi*.

This presentation will use a type of card-present fraud as an example to show how behaviors described can be detected through visualization of ATM datasets that are already being collected as part of standard operating procedure. Visualizing this data allows the human user to identify anomalies and use this information to contribute back to the design of effective algorithms to mitigate the threat. The objective of this form of data visualization is to identify and study criminal *modus operandi* in complex datasets and then use this information to design realistic automated rule sets for use in real time analytics tools. Incorporating visualizations into fraud control frameworks will help design new tools and mature existing tools and, thus, will be a positive

step forward for practitioners in the cyber crime prevention field. While there is much work remaining in this problem domain, this presentation demonstrates how anomalies can be identified and then coupled with the behavioral characteristics in context. The resulting information can be used to guide the evolution of analytical tools to help protect our digital assets.

Visualization, Digital Forensics, Fraud

B33 Assessing Skin Detail of the Dorsal Surface of the Hand: A Comparison of Methods

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After attending this presentation, attendees will have an understanding of two different techniques for segmenting the dorsal surface of the hand for use in image analysis. A comparison of the two methods will generate a greater awareness of the process of evaluating skin features commonly found on the dorsal surface of the hand and used in forensic image comparisons.

This presentation will impact the forensic community by assessing two different grid systems that may be employed in the course of a forensic image comparison. By knowing the strengths and weaknesses of each system, an examiner will be able to conduct a more thorough analysis when attempting to identify an individual through an image comparison.

The skin features examined in this study include: freckles, moles, sunspots, scars, and any other features of de-pigmentation or hyper-pigmentation. As these skin features vary in appearance, both in spatial distribution and geometric shape, each characteristic may be a potentially valuable resource when conducting comparisons to establish individuality. While such marks have been employed in facial recognition, hands are another area of the body that may be captured in images of forensic interest.

Two methods are assessed in the current study; both are grid systems that segment the dorsal surface of the hand into numerous regions. The first method, presented by Malone, includes 14 regions.[1] The second method, presented by Macdonald-McMillan, includes 24 regions.[2] Both methods use anatomical landmarks to divide the dorsal surface of the hand into its respective regions. The regions are then used to determine the location and spatial relationships between skin features. It is hypothesized that the method with more regions will generate more unique results, but an additional aim of the research is to determine the forensic practicality of each method. Depending on the situation, it may be more or less useful to have a greater number of regions to examine.

Each of the hand segmentation techniques was applied to a collection of 233 images. These images of hands were collected between the years 2003 – 2005, as part of the Computer Vision Research Lab Biometric Data Set at the Department of Computer Science and Engineering, University of Notre Dame. Each method was able to accurately quantify the features found in each of the regions generating descriptive statistics on the distribution and frequency of such skin features. Once the images of hands were assessed by both methods, the results were statistically compared. The advantages and disadvantages of each method were then examined.

Through this comparison of methods, an examiner is better equipped to perform photographic comparisons of the dorsal surface of the hand and to defend his or her conclusions in the courtroom. With the abundance of digital images and the potential for skin features to be examined, more research is required to establish consistency within the field of image comparisons.

*The opinions or assertions constrained 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.

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Image Comparison, Hand Identification, Image Analysis

C1 The Role of Nanotechnology in Forensic Investigations: Application of SEM in the Reconstruction of Crime Scenes

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The goal of this presentation is to underline the important role of the Scanning Electron Microscope (SEM) in forensic investigation and its usefulness in scene investigation in correctly describing the events.

This presentation will impact the forensic science community by describing SEM's potential application in forensic pathology.

In the last several years forensic pathology has increased the use of new instrumental methods of investigation. The use of nanotechnology increased in importance, particularly, the utilization of SEM.

Literature has highlighted the use of these methods, especially in the evaluation of the presence of organic and inorganic materials. These techniques are mainly used for analysis of terminal ballistics, for land surveys, and commodity surveys in the reconstruction of the crime scene. The purpose of these techniques is to provide isolation of the materials and to evaluate their eventual origin.

In this study, investigations were used to determine the compatibility of human injuries found on a cadaver with any harmful tools found at the crime scene. The purpose of this study was to detect the importance of applying these methods in the reconstruction of the crime scene by comparing the materials found in tissue organs with those recovered at the crime scene.

The case report examined a 50-year-old man found dead at work. The forensic investigation included: crime scene analysis, autopsy investigations, toxicological and histological investigations, and SEM analysis. On the scene investigation, the victim was found lying on the ground next to a cart used to transport granite blocks. On the cart were many fragments of granite. The external inspection of the body noted bleeding from the ear canals and a major injury to the right side of the face. The internal examination of the body found cranial and facial fractures and brain hemorrhages. The autopsy confirmed the presence of a serious head injury contributing to death. Histological examination showed extensive areas of cerebral hemorrhage. The external cause of injury was due to a macroscopic object compatible with different offensive tools recovered in the survey. The external characteristics suggested a crushing injury from a sheet of granite, which was not confirmed, as the body had been found, then moved to another location.

Crushing injuries are characterized by multiple, diverse, and multipolar marks. They are represented by bruising, abrasions, lacerated and contused wounds, fractures of skeletal segments, and the bursting of internal organs. In crushing by a granite slab, it is important to consider the damage caused by the surface of the plate and its weight. In this case, a section of granite and three cement fragments recovered at the crime scene were taken. At autopsy, six samples of skin tissue were collected. All samples were fixed in liquid nitrogen, stored at -80°C, and analyzed by a Microprobe Energy Dispersive (EDAX) mounted on an SEM. This analysis noted an overlap between the elements found in the granite (carbon: 28%; chromium: 63%) and tissue samples taken from the areas of injury. The overlap of data is not noted for the other materials that constitute other possible harmful tools (cement). Therefore, this technique allowed reconstruction of the crime scene, traced the lesions found on the cadaver to the tool (granite slab) recovered at the scene, and, importantly, attributed responsibility for the event.

Nanotechnology, SEM, Scene Investigation

C2 *Caveat Emptor*: What's a Residential Heating Oil Tank Owner to Do?

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After attending this presentation, attendees will understand the pitfalls faced by a homeowner with an underground storage tank who tries to do the right thing.

This presentation will impact the forensic science community by pointing out the non-technical perspectives to the issue of residential underground storage tanks.

You've lived in Westchester County, New York, for 10 years, and it's time to move back to Pennsylvania. So, you put your house on the market, find a buyer, and work out a sales agreement that includes performing a leak test of the Underground Storage Tank (UST) that has reliably held your heating oil since you moved in, and which, for the record, passed a similar leak test when you moved in. This time, though, it fails the test.

It's an old tank, so you decide to avoid potential future liability by removing it and replacing it with an above-ground tank in the garage. You call the contractor recommended to you by the company supplying your home heating oil. You are told it will cost you $2,800 to remove the UST and install an above-ground tank in the garage, and you pay half up front. The contractor comes out and digs a shallow ditch around the top of the tank. Now you are told there is discolored soil, and, based on a "sniff test," leaking oil was likely. The contractor collects a soil sample and tells you it will cost you an additional $3,000 for them to take care of the (alleged) contamination.

What? This is starting to feel uncomfortable, so you call your sister, who happens to be an environmental consultant. On her advice, you contact the New York State Department of Conservation (DEC) and learn that DEC had concerns about possible contractor irregularities related to UST work in Westchester County. *What?* The DEC representative comes to your house on the scheduled day for your contractor's next visit, but is left hanging when the contractor does not show. He talks to your contractor about the work on the phone and tells you the contractor is competent, but he can't come back the next day when the contractor has promised to be there. *What?!*

The contractor does show up the next day, and excavates down to the bottom of the tank. It is clear the tank is intact, and there is no evidence of contamination, by sight or smell. You are now told you will get your $3,000 back. The oil remaining in the UST is removed and transferred to the new storage tank in the garage. The contractor collects a soil sample from the excavation, re-buries the now-empty UST, and promises to return with the laboratory analysis results. *Whew … maybe it'll be okay after all.* Except that now he won't return your calls, and you can't proceed with settlement on the sale of your house.

Now you contact the Westchester County Department of Health (DOH) and tell your story, only to learn that the contractor has not followed their rules for this type of work. Settlement is delayed more

while you wait for your contractor to provide the necessary paperwork and do things right.

When he finally returns to actually remove the UST, you are there, too, with your environmental consultant brother-in-law and an inspector for the Westchester County DOH. Now you learn that the only reason the UST failed the leak test was because the original, but long-since inactive, fill pipe had never been properly disconnected. There was never any problem with the UST.

By the time the contractor files all the necessary paperwork, a 2-week project has dragged on for two months, costing you, among other things, two additional mortgage payments. You have not been reimbursed the additional $1,600 you paid, nor have you seen any laboratory results. Now what?!

We've all gotten the runaround at one time or another—dealing with health insurance companies is but one example. For the average homeowner, environmental issues are even harder to navigate. *Did it really have to be this hard? How much worse might it have been if there weren't two environmental consultants in the family?* Let the buyer beware.

UST, Residential, NYSDEC

C3 Combustible Dusts and Dusts From Combustion

James R. Millette, PhD, MVA Scientific Consultants, 3300 Breckinridge Blvd, Ste 400, Duluth, GA 30096*

After attending this presentation, attendees will understand how to identify combustible dusts and particles generated by combustion using microscopy tests.

This presentation will impact the forensic science community by providing information about combustible dusts that is an important safety concern for investigators entering dusty areas and useful forensic data in the study of explosions, fires, and sooty particulate.

When forensic investigators enter an enclosed area in a dusty building, they must be aware of the potential for a combustible dust explosion. The Occupational Safety and Health Administration (OSHA) provides a convenient poster that lists 116 combustible dusts divided into six categories: agricultural products (including corn starch), agricultural dusts (including garlic powder and raw yucca seed dust), carbonaceous dusts (including petroleum coke and pine soot), chemical dusts (including ascorbic acid and sodium stearate), metal dusts (including aluminum and iron carbonyl), and plastic dusts (including acrylonitile and epoxy resin).[1] The identification of an unknown dust as one of the OSHA 116 can usually be done by an experienced analyst with one of two microscopy tests. The shape, appearance, and optical properties as determined by Polarized Light Microscopy (PLM) are sufficient to identify many of the agricultural powders. The X-ray elemental analysis performed in the Scanning Electron Microscope (SEM-EDS) can determine which metal dust is present and the Fourier Transform Infrared Microspectrophotometry (FTIR) and Raman microscopy are capable of identifying the plastic dusts. Combinations of the four microscopy techniques (PLM, FTIR, Raman, and SEM-EDS) are used to identify the carbonaceous and chemical dusts. The identification of combustible dusts is important for both the safety of the investigators on-site and for the forensic experts reconstructing explosive or fire events.

Spontaneous combustion of agricultural materials can occur when bacteria slowly decompose the materials, producing heat. When the internal temperature reaches 185°F (85°C), hot spots and pockets may be expected. Flames will likely develop when these pockets come in contact with the air. Non-biological mechanisms may be involved with non-agricultural dusts. Pyrite oxidation has been reported as a cause of spontaneous ignition in old coal mine tailings.

After dusts have burned, microscopy tools are also used to characterize and identify the combustion products. Char particles, a product of partial combustion or a low-temperature situation, retain morphological characteristics of the original dust particles. Aciniform soot and flyash are the products of higher temperature combustion where the chemical elements of the original dust particles have reformed after being vaporized. The combination of a clear tape lift attached to a glass microscope slide and a wipe sampler is the preferred collection media set for soot testing. The clear tape lifts by themselves are helpful for screening purposes, especially to identify mold, biofilm, or other biological growth, but it is difficult to remove the particulate for other testing when a fuller analysis is in order. For instance, the finding by light microscopy of dark, opaque, aggregated particles on a tape lift suggest aciniform soot may be present, but this can only be confirmed with transmission electron microscopy as the diameters of the primary carbon particles in aciniform soot may be in the 30 – 40 nanometer range. The ASTM Standard D6602 is a method for identifying carbon black, an engineered aciniform carbon soot, that can be used to identify combustion dust particles in a variety of settings.[2]

References:

1. www.osha.gov/Publications/combustibledustposter.pdf (last accessed on 7/24/2012).
2. ASTM D6602 - 03b(2010)e1 Standard Practice for Sampling and Testing of Possible Carbon Black Fugitive Emissions or Other Environmental Particulate, or Both. ASTM International, West Conshohocken, PA, 19428-2959 USA

Explosive Dust, Soot, ASTM D6602

C4 High-Throughput Environmental Forensic Investigation to Identify Contamination Sources of Polycyclic Aromatic Hydrocarbons in a Stream

Melinda Pham, BS, and Frank Dorman, PhD, 107 Whitmore Labs, University Park, PA 16802*

After attending this presentation, attendees will understand how to prepare and clean up various samples prior to Gas Chromatography/Mass Spectrometry (GC/MS) analysis for environmental forensic needs. The sample clean-up procedure to be discussed is based on USEPA 3640A, but the procedure has been modified into an automated method using J2 Scientific's automated sample preparation instrument, PrepLinc. Attendees will also learn how to identify contaminants in a complex ecosystem through a discovery analysis. Lastly, attendees will learn a technique on how to identify possible sources of contamination via receptor model called Positive Matrix Factorization (PMF).

This presentation will impact the forensic science community by demonstrating an alternative automated sample clean-up method that would require less manual labor and an increase in sample throughput. The sample clean-up method will employ Gel Permeation Chromatography (GPC) and automatic concentration via an AccuVap. The forensic community will also learn how to identify sources of contamination and the contribution of those sources in sediment samples. This will be beneficial for differentiating natural and anthropological contamination sources.

Modern society has increased the usage and varieties of organic compounds for various benefits; however, there can be a negative side of the use of these same chemicals—environmental exposure. The "contaminant" may be transported great distances through the ecosystem before detection. In addition, once detected, the compounds may undergo significant degradation, metabolism, or other processes termed "weathering." This may make the identification of the original source of the pollution more difficult. It is the identification, quantification, and determination of the source or sources of environmental pollution that largely comprise the science of environmental forensics, and the subject of this presentation. The organic contaminants of interest in this study are Persistent Organic Pollutants (POPs) like Polycyclic Aromatic Hydrocarbons (PAHs).

PAHs are organic, atmospheric pollutants created through the incomplete combustion of fossil fuels like coal and petroleum. These

airborne pollutants can travel a great distance before depositing onto surfaces via wet and dry deposition. For this presentation, PAHs deposited into and around a nationally recognized fishing stream, Spring Creek, are investigated. PAHs are of environmental concern because of their persistent, bioaccumulative, and carcinogenic properties, thus, identifying and monitoring the contributions of PAH sources becomes important.

The PAH emission sources have distinct signatures, but the complex environmental matrix interferes with the analysis. To eliminate interfering compounds in the sediment samples prior to analysis, the automated sample clean-up procedure will be presented. The cleaned samples are then analyzed via GC/MS for identification and quantification.

Besides matrix complexity, sediment samples contain a collection of PAHs from natural and anthropological sources. Thus, differentiating between the two will be beneficial for environmental forensics application. Field sediment samples from Spring Creek are analyzed to identify PAH sources and their apportionment. The PAH emission sources and their contributions are identified using a receptor model called PMF. PMF is a multivariate statistical tool used to identify contamination sources and the contribution of each source in a given sample. PMF was chosen for this investigation because of the ability to assign experimental uncertainties to individual data points. Secondly, solutions are constrained to non-negative values, thus, overcoming limitations found in other, current receptor models like Principle Components Analysis (PCA). This work specifically discussed in this presentation may be applied to numerous other forensically relevant samples, and this will also be discussed.

PAHs, Forensic, PrepLinc

C5 Environmental Chemistry

James S. Smith, PhD, Trillium, Inc, 28 Graces Dr, Coatesville, PA 19320-1206*

After attending this presentation, attendees will have a better understanding of a chemist's role in environmental litigation.

This presentation will impact the forensic science community by focusing on the expert's qualifications instead of the general area of competence.

The essence of the litigation is based on forensic environmental chemistry dealing with questions such as:

- Who released the chemicals of concern (i.e., those substances perceived to harm human health or the environment)?
- How did the release occur?
- What manufacturing process or function produced the contaminant(s)?
- When did the releases occur (age-dating environmental contaminants)?

The answers to these questions must be found in the data from the site investigation of the contaminants in the various matrices, the fate and transport of the chemicals in these matrices based on peer-reviewed literature, and the fundamentals of chemistry and physics. The forensic scenario that is built by the expert witness should be logical and thoroughly based on the documents provided in the case, the chemical data that was obtained during the site investigation and that has been provided, and the chemical literature.

A case in point concerns chemistry and an environmental matrix such as groundwater. Is a chemist allowed to testify about chemicals in groundwater including their fate and transport? The attorney stated during *voir dire* of a chemist that fate and transport of chemicals in groundwater can only be addressed by an expert hydrogeologist. A chemist is not capable of determining the direction of groundwater flow nor its velocity. The use of tracer chemicals is not in the chemist's toolbox for the determination of the source of groundwater contamination nor where chemicals are transported by groundwater. The fate of chemicals in groundwater is for the hydrogeologist to interpret and opine upon. A chemist can discuss chromatography but cannot opine on the retention of chemicals in an aquifer.

A judge as a gatekeeper must decide if a chemist can or cannot testify at trial about the fate and transport of chemicals in groundwater. This is a new question and it does not consider the expert's qualifications but does consider the definition of the area of expertise.

Environmental Chemistry, Fate & Transport, Hydrogeology

C6 Scanning Electron Microscopy — Back to Basics

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After attending this presentation, attendees will gain insight into the importance of SEM detector geometries and their impact on data produced by a Scanning Electron Microscopy-Energy Dispersive X-ray Spectrometry (SEM-EDS) system.

This presentation will impact the forensic science community by demonstrating basic SEM-EDS detector geometries and showing how errors in Energy Dispersive X-ray Spectrometry (EDS) spectral acquisition can be avoided.

Scanning Electron Microscopy (SEM) became commercially available around 1965. Many improvements have been made since the early commercial instruments, allowing the SEM to have a presence in many laboratories and classrooms that use and study materials. Adding EDS to the SEM allows the user to quickly obtain the elemental composition of a material. Due to the advances in SEM-EDS hardware and software, very little instruction is typically required to train an individual in the operation of an SEM-EDS system. In fact, SEM-EDS is probably the easiest microscope to use in the laboratory.

Most of the operations for sample chamber evacuation, electron gun alignment, focusing, image contrast and brightness adjustment, and EDS data acquisition have become automated, requiring only the press of a button. Images and EDS spectra, once obtained, can be dropped into word processing, spreadsheet, or presentation software with a simple click and drag of a mouse…or the touch and drag of the "touch screen" that dominates today's telephones and tablets. Data acquisition using an SEM-EDS system has progressed to the point where the user, or the "human" interface, has become the slowest interface in the SEM-EDS system. Software algorithms have been and are being produced to analyze tremendous amounts of data that can perform iterative queries and produce groups of elements or show associations of elements (phases) during an SEM-EDS analysis that may otherwise be overlooked by the human interface. All of the automation, software, and user interfaces that have been designed to aid the microscopist in his (or her) analysis of a given material are both useful and welcome. The basic geometry of SEM-EDS systems and the electron beam-specimen interactions remain relatively unchanged with the low vacuum capability of modern SEM-EDS systems being but one exception.

SOPs and recipes have put the SEM-EDS within the reach of even the most novice user. These management tools enable laboratories to train individuals and replace individuals quickly so the laboratory remains productive. Unfortunately, no SOP or recipe, no matter how thoroughly written, can guarantee that the data produced is reliable. Fundamental concepts and knowledge of the basic geometry of the SEM-EDS system is essential to ensure the data produced is reliable. A useful approach to understanding the basic detector geometry of any SEM-EDS system is to think of the SEM imaging monitor as a clock face. Locate the EDS detector on the clock face by inspecting the exterior of the microscope chamber and determine where the EDS detector is. Is the EDS detector at 10 o'clock? Is it at 12 o'clock? Locate the secondary electron detector and any other detectors that are part of the SEM-EDS system. Knowing this information will help in assessing any elemental data produced when un-even non-flat specimens, also called "everyday specimens," are analyzed. The interaction of the primary electron beam with the sample and the line-of-site trajectory of the X-rays produced can affect the quality and reliability

of any EDS data produced. Examples of EDS data produced from specimens that are analyzed by SEM-EDS will demonstrate the effects of low vacuum, non-flat specimens resulting in X-ray fluorescence and absorption and general, basic SEM-EDS practices that will aid the SEM-EDS user in the analysis of everyday specimens.

SEM-EDS, Low-Vacuum, Elemental Analysis

C7 Detection of Drugs on Fingermark Residue Using Nanoparticle-Based Fingerprint Powders Using SALDI TOF-MS/MS Technique

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After attending this presentation, attendees will have an understanding of the dual nature of fingerprint powders to develop and detect drugs on fingermark residue using Surface Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (SALDI TOF-MS) technique.

This presentation will impact the forensic science community by providing results of trials and experiment conducted using nanoparticle-based ARRO MS latent fingerprint development powder. The presentation will provide experimental evidence using the powders in enhancing the definition of the developed fingerprint and to directly analyze the drugs on the lifted fingerprints.

The presentation will provide experimental evidence of the work performed to visually identify fingerprints using nanoparticle-based fingerprint powder. The developed print can then be lifted using lifting tape and can then be directly analyzed to identify the chemical constituents present within the fingerprints which might include any contact residue of the drug handled and/or metabolites of the drugs excreted through the pores of the skin. A range of drugs including therapeutic (paracetamol, aspirin, and lisinopril) and illicit (cocaine, methadone, and amphetamine) drugs were identified on fingermark residues (with or without cyanoacrylate fuming) after developing with nanoparticle-based latent fingerprint powders (ARRO SupraNano MS black magnetic powder) and analyzing using SALDI TOF-MS technique. Peaks were identified for all the drugs under study for the lifted fingerprints dusted with MS black magnetic powder and with Dihydroxy Benzoic Acid (DHB), a commonly used maldi matrix agent. Results were compared for lifted prints with or without cyanoacrylate fuming prior to dusting. For fingerprints dusted with MS black magnetic powder, in addition to the identification of the drugs, the developed fingerprints were highly defined, revealing more details of the fingerprints, due to the smaller diameter and the sphericity of the nanoparticles.

Method: Fingerprints spiked with therapeutic (paracetamol, aspirin, and lisinopril) and illicit drugs (cocaine, amphetamine, and methadone) were deposited on a clean glass slide, followed by dusting with MS black magnetic powder. The prints were then lifted using a standard fingerprint lifting tape and were stuck to a maldi target plate with the adhesive side facing up. Analysis was performed using Shimadzu Axima Performance Maldi TOF-MS after calibration using small molecule calibration mixture for the masses ranging between 132 to 607 using positive ionisation mode. Reference spectra for the drugs under study were obtained by spotting 1µl of the drug standard (10µg/ml) along with 1µl of 10mg/ml of DHB and analyzing it in positive mode. For the lifted fingerprint, laser shots were randomly fired across the fingerprint area and the average profile of the spectrum was collected using approximately 400 shots for each fingerprint region. Similar procedure was conducted for the spiked fingerprint without any dusting agent and with DHB dusting.

Results and Discussion: The nanoparticle-based SupraNano MS black latent fingerprint powder gave superior definition, exhibiting 3rd level ridge detail with high contrast against the background. The smaller diameter and the sphericity of the nanoparticles is the prime reason for the enhanced performance of the latent fingerprint powder.

SALDI analysis of the lifted fingerprints dusted with DHB and MS black magnetic powder gave M+1 peaks for all the drugs studied. However, for fingerprints dusted with MS black magnetic powder, sodium and potassium adducts of the drugs were also identified along with the M+1 peak for most of the drugs. Nanogram levels of the drugs were able to be identified on the lifted fingerprints dusted with MS black powder. The carbon black-doped silica nanoparticles used as the active ingredient in the latent fingerprint powder aids in the ionisation process, thereby enhancing the signal intensity even at trace level concentration.

With the results obtained, it can be concluded that the MS black magnetic powder can be used for the dual purpose of developing the fingerprint (with or without cyanoacrylate fuming) for superior definition and for directly detecting the chemical constituents present within the fingerprint using SALDI TOF-MS.

Nanoparticle, Fingerprint Powder, Drug Analysis

C8 Two-Dimensional X-Ray Diffraction for Forensics and Archaeology

Bob B. He, PhD, Bruker AXS, 5465 East Cheryl Pkwy, Madison, WI 53711*

After attending this presentation, attendees will gain an understanding about the basic concept and recent advances of two-dimensional X-ray diffraction, including the key technology, configurations, and applications relevant to forensics and archaeology.

This presentation will impact the forensic science community by introducing the two-dimensional X-ray diffraction as a powerful material characterization tool as well as the advantages for forensics and archaeology. This presentation will provide experimental examples both in general material research and forensic studies.

Compared to the conventional X-ray diffraction, the two-dimensional diffraction pattern contains not only the diffraction intensity distribution in two-theta direction based on the Bragg law, but also the diffraction intensity distribution along the gamma direction (along the diffraction ring).[1] The intensity distribution and two-theta variation along the gamma direction can reveal the strain, crystallite orientation distribution, and size. The diffraction patterns, appearing identical with the conventional X-ray diffraction, can be significantly different if observed with two-dimensional diffraction. Therefore, two-dimensional X-ray diffraction can distinguish two samples with identical chemistry content and phase as long as they were formed at different conditions. Two-dimensional diffraction patterns are also easy to compare, evaluate, and present to the courtroom.[2]

The recent advances in two-dimensional X-ray diffraction have significantly benefited forensics and archaeology. The brilliant X-ray sources can deliver highest X-ray flux for fast data collection and deal with small sample volume, weak diffraction, and low concentration. The point focus preferred with the area detector is very suitable to point the X-ray beam to the interested area of evidence and controlled substance. The photon-counting area detector with a large detection area, high sensitivity, high resolution, and near-zero background noise can reveal the phase, crystal structure, crystal particle size, and crystal orientation from a very small amount of the sample. Typical analysis for forensics and archaeology involves identification of materials and structures from a small amount or a small area of samples, also referred to as microdiffraction. In order to preserve the original evidence and art, the analysis must be done non-destructively and without sample treatment. Two-dimensional X-ray diffraction is an ideal, non-destructive, and highly sensitive analytical method for examining samples of all kinds, such as metals, polymers, ceramics, soils, coatings, paints, biomaterials, and fibers for forensic science and archaeology. Sample alteration or treatment is typically not necessary and the data collection can be repeated many times without any damage to the sample. Two-dimensional diffraction patterns contain abundant information and are

easy to observe and explain in the courtroom. This presentation will cover various applications used for forensics and archaeology analysis. Experimental examples for general materials characterization, including phase identification, texture and microstructure analysis, and case study examples in forensics and archaeology are also given.

References:

1. Bob He, *Two-dimensional X-ray Diffraction*, John Wiley & Sons, 2009.
2. W. Kugler, X-ray diffraction analysis in the forensic science: the last resort in many criminal cases, *Advances in X-ray Analysis*, 2003, 46, 1-16.

XRD, Microdiffraction, Area Detector

C9 Use of High-Definition Survey (HDS) Laser Scanning in Forensic Engineering

Steven M. Schorr, PE, DJS Associates, Inc, Forensic Engineering Services, 1603 Old York Rd, Abington, PA 19001*

After attending this presentation, attendees will gain knowledge regarding the utilization of High-Definition Survey (HDS) laser scanning. Engineers are now able to, cost-effectively and with unprecedented accuracy at the speed of (laser) light, collect millions of measurements and create a 3D world.

This presentation will impact the forensic science community by illustrating how HDS laser scan data is collected; how the data is utilized in the industry; how the data and its by-products have been presented in court; the basic costs; and the strengths and weaknesses of the technology. This presentation will also focus on the unique issues that may arise when HDS laser scanning is utilized in the forensic field, including how to prepare for the expected challenges to the data collection process and accuracy.

We view our everyday world in 3D so why should we not be able to view and analyze our collisions, incidents, accidents, and other types of occurrences in the same manner? With the advent of state-of-the-art technology in data collection, the use of HDS laser scanners now makes this possible. Through the utilization of this cutting-edge laser scanning technology, engineers are now able to, cost-effectively and with unprecedented accuracy at the speed of (laser) light, collect millions of measurements and create a three-dimensional world. The quality of an engineer's analysis is directly related to the quality of the collected data, so this new technology is rapidly advancing the field of forensic engineering.

Traditionally, forensic engineers have utilized the same equipment used by professional surveyors. Though this equipment is still a valuable tool in the engineer's toolbox, its speed limitations and measurement recording technique has been far surpassed by laser scanning technology. Before laser scanning, the data collection process prevented engineers from taking more than a couple of measurements per minute. Now, engineers can record hundreds of thousands of measured points every minute. The measurements are recorded from laser light, which reflects from objects after being projected from a series of rotating mirrors. The scanner is indiscriminate in what objects it measures, meaning that precise measurements can be taken of just about anything. This relatively new technology is rapidly becoming more and more affordable to forensic professionals. The HDS laser scanners have the ability to quickly, accurately, and thoroughly collect 3D data for almost any type of incident and environment. The HDS laser scanners are routinely utilized to collect fresh, physical evidence at building collapses, bridge failures, vehicular collisions, and anywhere there is a need for precise 3D measurements. This is especially beneficial in capturing the scene as it was at the time of the occurrence, if the incident area will be changing. HDS laser scanning data is so beneficial that, even in its raw form, it can be utilized for critical data, measurements, and even exhibits. The mass quantity of recorded raw data collected from long-range scanners (frequently referred to as a point cloud) is accurate to within a few millimeters (approximately the size of a pencil eraser). In addition, because the raw data cannot be altered or modified in its initially recorded state, the data validation process with other engineers and investigators has been greatly streamlined. The 3D data is also a foundation for other engineering analyses, providing an accurate baseline data to effectively utilize scientific tools such as photogrammetry and camera-matching. The "point cloud" data can also be used to quickly and accurately create real-world, 3D models for use in demonstrative exhibits, like simulations and animations.

HDS Laser Scanning, 3D Measurements, Camera Matching

C10 Large Area Hyperspectral Imaging of Effluents as an Example of Technology Transfer

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After attending this presentation, attendees will learn about an airborne Hyperspectral Imager (HSI) developed for the government and the efforts to transition this device into the law enforcement arena.

This presentation will impact the forensic science community by addressing how not all laboratories doing research into new instruments/methods that have forensic applications need to be certified and the scientists performing this research need not be accredited forensic investigators for their research to be acceptable in court.

This presentation will present an airborne HyperSpectral Imaging (HSI) sensor as an example of technology developed by The Aerospace Corporation, a California nonprofit company that operates a Federally Funded Research and Development Center (FFRDC), for the federal government and the effort to transition the device into a forensic tool. This technology was first demonstrated to law enforcement and public safety personnel using a small, ground-based instrument at a court-supervised methamphetamine cook in Southern California in 1998 by the National Law Enforcement and Corrections Technology Center – Western Region (NLECTC-WR). The NLECTC-WR was a National Institute of Justice (NIJ) center run by The Aerospace Corporation. The technology uses Thermal InfraRed (TIR) spectroscopy to detect and identify hazardous chemical effluents such as such as those created by methamphetamine labs (methyl ethyl ketone, Freon, etc.) and Improvised Explosive Devices (IED) production.

A predecessor of the current instrument was flown over the debris of the World Trade Center to locate hazardous materials, such as asbestos, ammonia, etc. The current instrument is greatly improved and can acquire data over much larger areas and process that data much faster than its predecessors. The current MAKO system capability will be described in detail as well as the path forward for this technology. The effort to transition this technology to forensic applications will also be put forth.

The airborne nature of the MAKO instrument is an expansion of the concept of crime scene boundary. The scientific examination of the 9/11 Twin Towers destruction expanded the concept of the crime scene boundary. It was based on an increased scientific capability. This expanded capability through translational science is the core message of this paper. The improved metrics and capability of improved technology are described as forensic advances in this paper.

Questions raised about the legal ramifications of this and other advanced technologies will be put forth and a path forward to get answers will be discussed.

In addition, the presentation will describe short- and long-term plans for utilizing this and other tools developed by the government for military applications as real-time forensic tools to systematically improve, on a volunteer basis, security, safety, and quality of life.

1. Integrate with the national agenda of All Hazards Preparedness. National preparedness is a quest.
2. Integrate with internationally available satellite systems, such

as weather satellites and GPS, for accurate time and location of effluent events as well as their possible movements.
3. Develop synoptic displays applicable for first responders and non-scientific experts.
4. Develop concepts of operations for real-time forensics utilizing these tools.
5. Partner with law enforcement and public safety agencies to license/commercialize.
6. Provide training. Help the public differentiate fact from fiction.

Technologies, such as the HSI instrument presented here, will continue to be developed by institutions that are outside the realm of accredited forensic laboratories by scientists who are not accredited forensic technicians. There needs to be a method to transition these technologies into the forensic realm by proving their utility on actual casework and in the courts before the accreditation process can begin.

Hyperspectral, Imaging, Accreditation

C11 Crashworthiness Simulations of Glass Mat Thermoplastic (GMT) and High Strength Steel (HSS) Sedan Bumper Beam Designs Using Explicit Finite Element Analysis

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After attending this presentation, attendees will be exposed to how explicit finite element analysis can be used to investigate deficiencies in crashworthiness, as well as how it can be used to suggest superior alternative designs. The state-of-the-art in bumper design will be discussed. The importance of materials testing to failure investigations will be emphasized through examples of specific cases where it proved valuable.

This presentation will impact the forensic science community by showing and recognizing shortcomings of some current bumper designs as well as broadening their exposure to tools that can be used to investigate crashworthiness in general. Identifying these problems and educating others about it will serve to create a driving force toward solving them.

Nearly all front and rear bumpers on modern sedans use either a High-Strength Steel (HSS) bumper beam or a Glass Mat Thermoplastic (GMT) composite bumper beam. GMT bumper beams are better at absorbing energy in low speed crashes than HSS and perform better in pedestrian protection tests; however, at higher speeds they fracture. GMT and HSS designs were investigated for crashworthiness at high and low speeds using explicit finite element analysis.

Three-dimentional models were created of the frame and bumper structural parts located at the front of one sedan and the rear of another. The 3D models were created using a portable Coordinate Measuring Machine (CMM). Using the CMM, data was collected from two production sedans. The data was used to create very accurate 3D models of the frame structures that contributed the most interaction in the collisions studied. The portion of the sedan that was not expected to deform or contribute much interaction other than mass and inertial properties was modeled with a sled based on the Advanced European Movable Deformable Barrier version 3.10 specification. The sleds were given the inertial properties of the modeled sedans.

Samples of bumper materials and frame materials were extracted and tested. Tensile testing was performed on all steel samples. Fourier Transform Infrared Spectroscopy (FTIR), tensile tests, flexural tests, and out-of-plane shear testing was performed on Original Equipment Manufaturer (OEM) and aftermarket GMT bumpers. Mechanical properties obtained from the tests were incorporated into the finite element model. Significant differences were found between the manufacturers' mechanical material property specifications and those obtained from testing the material. The effect of these differences was investigated with the Finite Element Analysis (FEA) model.

The sedan with the modeled front bumper was placed behind the sedan with the modeled rear bumper. The front sedan was at rest, while the rear sedan was given varying initial velocities and allowed to impact the front sedan. Simulations were performed with changes made to the front sedan's rear bumper geometry and material and results were compared. Vehicle velocity versus intrusion was plotted and the energy absorbed by the different bumper designs was compared. Real world crash testing was performed using the two sedans which was used to validate the FEA model. All simulations were performed using the commercial finite element analysis software LS-Dyna.

It was found that actual mechanical properties of tested OEM GMT bumpers were significantly less than specified by the manufacturer and that this significantly reduced the bumpers' real world performance. It was found that at speeds over 5mph the GMT bumper beam fractured and completely separated, while the HSS bumper beam did not. This leads to significant differences in the energy absorbed by the bumper beam designs at speeds higher than 5mph. It was also found that the bumper design can affect whether the bullet vehicle is directed down and into the frame or up and into the trunk where little structure exists to absorb energy and keep intrusion out of the passenger compartment.

Crashworthiness, FEA, Bumper

C12 Breakage Characteristics of Glass Drinking Vessels and Consequent Injury Potential in "Glassing" Attacks

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After attending this presentation, attendees will gain awareness of typical breakage patterns of a representative selection of glass drinking vessels found in bars and pubs, and understand the particular conditions in which they occur.

This presentation will impact the forensic science community by providing a detailed account of the failure of drinking glasses, and thus giving an indication as to whether or not an alleged account of a glassing assault is likely to be valid or not.

The failure of glasses is dependent upon a complex function of: (1) flaws that are either inherently present or due to handling damage in/of the glass; (2) the glass' thermal history and, hence, the extent of residual stress present; (3) the magnitude and distribution of areas of individual stress concentrations due to loading; and, (4) the mechanical properties of the glass itself. Since nearly all commercial glasses are soda-lime silicates, the mechanical properties of glass can be assumed to have negligible effect.

Figure 1 shows a Finite Element Analysis (FEA) simulation of a pint glass subject to impact. Areas of high stress concentration are likely to serve as the origin of failure but more vulnerable areas of the glass (i.e., the rim) are likely to be the source of the most severe flaws. Failure occurs when the critical fracture toughness is exceeded. Where a large flaw is found, the energy necessary to cause fracture is low and a claim of accidental breakage and consequent injury may have merit.

The introduction of tempered glasses over the last two decades has led to an increase in glass safety by causing the glass to break into smaller and blunter fragments. A rapid cooling treatment of the glass surfaces from the glass transition temperature causes a mismatch of fictive temperatures throughout the glass wall thickness, leading to compressive stresses at the surfaces and a balancing tension stress in the core of the glass. Since glass will only fail in tension and usually from a surface flaw, the magnitude of compressive stress can be added directly to the inherent glass strength. The combination of the additional force required to cause fracture and the release of the residual stresses causes a far more dense fracture pattern.

However, the level of temper possible is dependent upon the thickness of the glass and it is found that thinner parts of the glass (i.e., closer to the rim) store far less stress and resulting fragments there are similar to that of annealed (residual stress-free) glass. This can be seen in Figure 2.

Not only does the level of residual stress vary up and down the height of the glass, but also around its diameter. This can be measured using an automated polariscope that interprets the degree of retardation of light due to stresses in the glass. A prediction of residual stress can also be made by analyzing the distribution of wall thickness, which is aided by the use of CT imaging.

This presentation will show a range of characteristic breakage patterns produced by varying types of glasses, and illustrate what factors must be incorporated into the production of a safe pint glass.

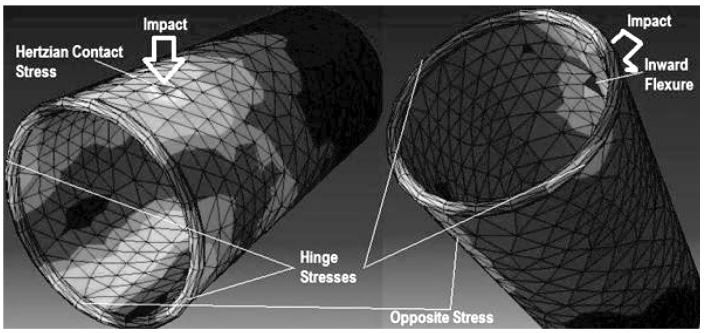

Figure 1 — Finite element simulation of stress distribution on a straight-walled pint glass due to impact. Left image shows the Von Mises stress distribution, right image shows first principal stresses.

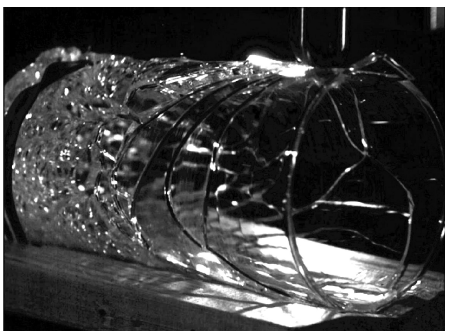

Figure 2 — A frame taken from high-speed video footage of a tempered glass fracture. Near the base of the glass, the toughening process is effective and the glass breaks into small shards. For this glass, toughening is less effective closer to the rim, resulting in larger and sharper shards.

Glass, Glassing, Fragment

C13 3D Characterization and Comparison of Fracture Surfaces

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After attending this presentation, attendees will become familiar with fracture characteristics from materials and fracture events.

This presentation will impact the forensic science community by answering the National Academy of Sciences (NAS) call in the area of fracture surface matching where traditional matching techniques become limited when macroscopic surface features are degraded, and to incorporate an engineering technique.

In 2010, according to the FBI's Uniform Crime Report, over 150,000 violent crimes were reported to have a knife or other sharp, cutting instrument involved. Conclusively matching two halves of a fracture surface can be crucial to a criminal investigation. For example, if a knife tip is found at a crime scene, proving that it belongs to the suspect's knife depends upon the expert opinion of the forensic examiner. At present, a visual pattern match of the two surfaces is the most common way to match fractured pieces. The 2009 National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward* calls for reforms and major changes to current forensic science practices. The goal of this project was to answer this call in the area of fracture surface matching where traditional matching techniques become limited when macroscopic surface features are degraded, and to incorporate an engineering technique with mathematically and statistically defendable error rates to fill this gap.

The goal of this study was to determine whether surface measurements could be used to conclusively associate two fracture surfaces. With the use of instrumentation (Direct White Light Interferometry (DWLI), Scanning Electron Microscopy, and Optical Microscopy), micron level measurements were gathered from the fracture surfaces of both the knife tip and the suspect knife. The measurements would then be used to compare the two fracture surfaces and determine if they were from the same fracture event. This new method seeks to assist the examiner by providing mathematical support for an exclusion, identification, or inconclusive determination concerning the tip and suspect knife.

In order to achieve this, a two-inch section from each knife in a set of was broken off. Each knife now consists of a tip and a base. For the experiments, each knife base was assigned a random code consisting of a letter followed by two numbers to differentiate this set of samples from others. The samples were all examined and matched to the other half using an optical microscope simulating conventional forensic fracture matching.

Direct White Light Interferometry was utilized to scan the samples in three predetermined zones of the fracture surfaces. Fast Fourier Transform algorithms were used to process the topographical surface feature data to convert the data into distinct average frequencies for each surface. An algorithm was used to compare the average frequencies of each surface pre- and post-exposure to delineate match results.

The fracture surface topography measurements were collected using the DWLI. To capture the range of surface features, a magnification of 20x was chosen. The field of view at this magnification of 20X is 0.55mm by 0.55mm, representing only a portion of the entire surface during the DWLI scan. To obtain an accurate representation of each fracture surface, three scans are taken, each in a different region of the fracture surface. The DWLI records the scan in nanometers, and the output is a three-column matrix, where the first two columns designate the pixel and the third column is the height at that pixel.

The matrix is run through an algorithm which uses the Fourier Transform to compare the mean frequency of each fracture surface. The difference between the mean frequency of two fracture surfaces indicates if the surfaces are a match or not. With the narrow height range parameters used to measure the fracture surfaces on the instrumentation, the software is used to discriminate between samples. Comparisons of fracture surface feature frequencies between samples will be discussed in terms of a quality of match for a known match versus non matches.

Fracture, Steel, Interferometry

C14 Examination of Microstamped Cartridges

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After attending this presentation, attendees will gain an understanding of the durability and reliability of microstamped firing pins. The quality of the alphanumeric identifier will be discussed as well as how the gear code on microstamped firing pins can be deciphered. How the transfer of the identifier varies between different guns and ammunition will also be discussed.

This presentation will impact the forensic science community by providing results from a microstamped study, an area that is currently receiving a lot of attention. This presentation will add to research being carried out in ballistics by broadening the understanding of factors that affect the transfer of the alphanumeric identifier as well as a circumferential gear code and its decipherability.

Recently, firearm microstamping, which involves placing unique identifiers on the end of a firing pin that is then transferred to the primer upon firing, has been proposed as one method of improving forensic identification. Current microstamped marks consist of six to eight alphanumeric characters and a surrounding circular gear code, which is intended to confirm the alphanumerics. While a quick visual observation can determine which alphanumerics are clear, any distortion of the identifiers or sanding of the firing pin tip can make identification difficult. In these cases, the gear code could provide information that could fill in missing identifiers, or could replicate the whole alphanumeric code if it is illegible.

This study seeks to evaluate the transfer of gear codes from microstamped firing pins to a variety of ammunition types. The initial part of this study has already appeared in print.[1] Briefly, 1,000 cartridges were fired with three different semiautomatic handguns: a Sig Sauer P226, a Taurus PT609, and a Hi-Point C9. Microstamped firing pins for each gun were optimized with six alphanumeric characters. Of the 1,000 rounds, ten different brands of ammunition were chosen with a variety of primer types represented. The fired cartridges were then evaluated through a stereomicroscope and assigned a grade based on the number of legible alphanumeric identifiers. The poorest quality transfers from this study were chosen to be examined through Scanning Electron Microscopy (SEM).

Like the identifier evaluation in the previous study,[1] the Sig Sauer had the best transfer of gear code. The Taurus did not transfer its gear code well, and the Hi-Point transferred its gear code consistently, though only certain sections. Interestingly, the overall clarity grade was improved most, not from the gear code, but by simply changing the imaging technique. SEM allowed more of the identifiers to be clearly read, though some still were too smeared to decipher.

In addition to evaluating the poorest quality cartridges, the lowest graded clips (10 cartridges per clip) from each gun were also evaluated to examine the possibility of using parts of the gear code and identifiers to form a complete microstamp. As was the case in the earlier part of this study, the Taurus cartridges did not have good gear code transfer.[1] No gear code transferred beyond the second character; however, the identifiers did reveal the whole code. The Sig Sauer was the only gun that had every section of its gear code transfer at least once. The Hi-Point's gear code only appeared, at most, to confirm the first three identifiers.

In conclusion, this study investigated the transfer of the identifier and gear code in microstamped marks from three different guns. In no case was transfer universal. Poor transfer was also seen and, in the case of the gear code, occasionally contained errors. However, using both the gear code and the alphanumeric identifiers, the complete microstamped code could be deciphered in every case when using all the information contained on cartridges from a 10-shot clip. The identification of the alphanumeric was most easily facilitated by simply using SEM instead of a stereomicroscope for evaluation.

Reference:

1. L.S. Chumbley, J. Kreiser, T. Lizotte, O. Ohar, T. Grieve, B. King, & D. Eisenmann (2012). Clarity of microstamped identifiers as a function of primer hardness and type of firearm action. *AFTE Journal*, *44*(2), 145-155.

Microstamping, Gear Code, SEM

C15 Interpretation of Evidence Resulting From a Stabbing Event Through Single Jersey Fabrics

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After attending this presentation, attendees will learn about the physical properties of common T-shirt fabrics, the effects of laundering, how these properties influence the residual damage after a stabbing event, and the problems associated with laboratory simulations.

This presentation will impact the forensic science community by providing information on the importance of background knowledge in textiles when assessing and re-creating clothing damaged in stabbing attacks.

Stabbing is the most common cause of murder in Australia, and knitted T-shirts worn next to the skin are popular garments. The beneficial evidence gained from textile examinations in forensic science is widely acknowledged. Simulation experiments are often required to test conflicting scenarios within a homicide inquiry because fabrics from the stab attack cannot be used for simulation testing. Substitute fabrics of similar fiber composition and physical structure are then investigated using a suitable underlying body simulant. It is usually assumed that the garment from the crime scene has previously been worn and has thus been laundered multiple times. Simulant fabrics must, therefore, be appropriately pre-conditioned prior to testing. This field of forensics is currently being investigated from both forensic and textile perspectives; however, there appears insufficient emphases on the details of the physical parameters and properties of fabrics.

This study considers the physical condition of a commonly worn single-jersey fabric damaged by a stabbing attack when it is described as "new," "worn," and "old." The goal was not to present a standardized method for pre-treatment on all textile fabrics. A universal method for pre-treatment cannot be achieved since different textiles/garments have individual care procedures. A realistic Australian home-wash procedure was selected. To further degrade specimens to a level that cannot be achieved by simple domestic laundering, selected fabrics were subjected to a commercial stonewash. The intent was to identify how the physical condition of a fabric affected the residual damage inflicted by a stab penetration.

Knitted fabrics behave very differently from woven fabrics. Dyed and finished single-jersey fabrics were obtained from a local manufacturer. Pure 100% cotton knits of 140g/m^2 and 180g/m^2 were selected to determine if they behaved differently when stabbed with a given knife design and entry conditions. A 65%/35% blended polyester/cotton knit of 150g/m^2 was also evaluated.

Fabrics were laundered five times according to 5A of AS 2001.5.4-1987 and line dried between each cycle. All other fabric physical testing followed an appropriate Australian or International Standard method. The physical properties of the yarns, such as linear density, yarn twist, breaking strength, and elongation, were determined. For the fabrics, parameters such as mass per unit area, thickness, courses and wales, cover-factor, stretch and recovery, and bursting strength were determined. Dimensional stability and spirality of the knits were determined after laundering and stonewash pre-treatments. The fabrics were tested for their physical parameters and properties, in original state, after laundering, and after stonewash treatment.

One emphasis of this study has focused on re-creating the stabbing event. To produce realistic and consistent predictions, suitable human skin and tissue simulants were investigated. Due to its variability, the traditional porkbelly simulant was found to be unsuitable. Pickled

kangaroo skin placed over 10% per weight Vyse ballistic gelatin was established as an acceptable representation of the human body. Kangaroo skin also shares similar fibrous structure and directional properties to human skin. A test rig was developed that enables reproducible penetration impacts at pre-determined energies. Experiments were conducted on knitted fabrics placed over the kangaroo skin and ballistic gelatin. They were stabbed using a 20cm kitchen knife, the blade of which impacted parallel to the courses, wales, and diagonally.

As the simulation model for a human body contains no blood, a simple blood-droplet absorbency test was also undertaken on the fabrics. Commercial fabrics are subjected to a number of finishing treatments which can remain on a garment after purchase. T-shirts often have a softener applied which dramatically affects their absorbency. Extractable matter was removed from the fabric before and after laundering to confirm the quantity of treatment. A modified droplet absorbency test using horse's blood at 37±1°C determined the effects of laundering. Macroscopic and microscopic examination of the stabbed specimens revealed differences between the physical damage. These characteristics of damage were then compared to the physical parameters and properties of the fabric.

T-Shirt, Stab, Damage

C16 Comparison of Striated Marks From Slip Joint Pliers

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After attending this presentation, attendees will have a better understanding of the difficulties associated with quantitatively analyzing striated marks such as those produced by ~~a~~ the shear force seen when pliers are used for cutting wire. These marks are much more complex than those seen when using, for example, a screwdriver to mark a piece of lead. A statistical algorithm suitable for analysis of a simple mark has poor results when used to compare more complex marks.

This presentation will impact the forensic science community by showing there is an inherent difference in plier marks as opposed to simple linear toolmarks. It appears that entirely new methods and statistical algorithms will need to be developed in order to reliably characterize shear marks produced by pliers, bolt cutters, garden brush clippers, etc.

Studies have shown that a computer-based system developed at ISU works well when presented with striated marks, such as those made by a screwdriver, where the marks produced are a simple linear array of wide and narrow channels, which are, in essence, a negative of the surface of the screwdriver tip used to make them.[1] However, the problem becomes more difficult when the mark examined is less regular in appearance, such as those made by pliers. Although both are shear marks, it quickly becomes apparent when studying these markings that the plier marks are much more complex. The more complex marking results due to the nature of the cutting process. The cut is produced by the shearing action of two separate plier faces coming together to produce the cutting edge. Similar to screwdrivers, where each side is characteristic, yet different from, the other side, both sides of a plier cut are different. However, as a cut is being made, the cut material is subjected not just to the tip of the cutting plier face on each side, but also the material is dragged across the entire surface of the face during the cutting process. At this time it is highly probable that the original striated surface produced at the very edge of the cut will be altered/changed as the remainder of the plier face shears past it. The resultant mark consists not of a negative of the tip of the cutting face, but is a composite of the plier cutting face and deformation by the surface of the face as it travels through the material to achieve a complete cut.

To study the applicability of the current algorithm to more complex markings, fifty pairs of sequentially manufactured pliers were obtained. The pliers were as identical as possible, being taken directly from the manufacturing line and never used. Alternating sample cuts of lead and copper wire were made using the shear surfaces of the pliers. The cut surfaces of the samples were then scanned using an optical surface profilometer to obtain the surface geometry.

The resulting data files were cleaned to remove background noise, then compared using the statistical algorithm that had proven useful for the comparison of simple striated screwdriver tool marks. The comparisons with the algorithm were divided into three different groupings: known matches, known non-matches from the same pair of pliers (different sides of the cut), and known non-matches from different pairs of pliers. The results of the comparisons varied, and trends could be seen showing known matches had higher scores in general than known non-matches. However, the results are much less definitive than for the more striated marks. The effects of changing data collection locations for comparison as changing comparison parameters showed no clear trends for improvement. The details of these comparisons conducted will be discussed.

Reference:

1. L. S. Chumbley *et al.*, "Validation of Tool Mark Comparisons Obtained Using a Quantitative, Comparative, Statistical Algorithm," *Journal of Forensic Sciences* 55(4), 953–961, 2010.

Slip-Joint Pliers, Tool Mark Comparison, Statistics

C17 Seatbacks: Rigid or Yielding?

William H. Muzzy, BS, 1509 Porter Ave, Ocean Springs, MS 39564*

The goal of this presentation is to discuss the philosophy of seatback design, whether the automobile front seatback should yield rearward or remain upright during a rear-end collision. The answer will become apparent after the discussion.

This presentation will impact the forensic science community by showing how results of three case studies and prior research should provide seat designers and decision makers with information to improve seatback designs and regulations.

This presentation will demonstrate that automotive seat designers must consider human tolerance, not federal standards, when setting seatback yield strength.

The federal standard for seatback strength is set so low that a lawn chair can pass it. Two groups are divided on front seatback design to protect occupants from rear line-of-force impacts. One group propounds that seatbacks should yield rearward to minimize forces on the body and reduce whiplash. The other group advocates a strong seatback that stays upright and contains occupants in the seat.

Whole body tolerance to +Gx impact was established experimentally in 1958. Captain Beeding recorded 82.6g on his chest accelerometer when he decelerated from 32.8mph to stop with a peak acceleration of 40.4g.

Crash data recorders, installed in race cars in the 1990s, recorded +Gx accelerations of up to 110g when the cars impacted the hard retaining walls. Drivers survived with recoverable injuries.

Human tolerance to +Gx acceleration was established and confirmed with human tests and recorded crashes with the Indy race cars.

Three case studies will show a comparison between rigid and yielding seatbacks and answer the title's question.

Case One: Both southbound lanes of a divided parkway were backed up for $1^1/_2$ miles. At the front was a truck with a large load that had stopped to measure the height of an overpass. A 1999 four-door sedan was the last vehicle in line in the outside lane with a mini-van just in front of it. Both vehicles were almost stopped on a small bridge.

A 26-foot-long two-axle truck was traveling in the outside lane with the cruise control set at 70mph. The driver claimed he did not see the stopped traffic in front of him in time to stop and impacted the rear of the sedan. This collision caused the sedan to go airborne, impact the left

guard barrier, and rotate 180 degrees. The truck continued on, crashing into the rear of the mini-van, pushing it forward.

The rear half of the sedan was severely crushed up to the B-pillar where the truck rode up over the sedan's bumper. This sedan was equipped with energy-management front seats and the seatback remained upright. The female driver got out of her car with minor injuries.

The mini-van's rear gate and quarter-panel were pushed up to the rear wheel on the left side while the right rear quarter-panel was barely deformed.

The female driver's seatback yielded rearward until it hit the rear seat cushion. She ramped up the reclined seatback, receiving a left occipital skull fracture, subarachnoid hemorrhage with possible coup-counter coup lesion, closed head injury, and left forearm fracture.

Case Two: A large 2002 four-door SUV was traveling at highway speed when the left rear tire detreaded. The vehicle swerved to the right and rotated clockwise as it left the right side of the highway. It slid off the highway until it hit a tree just behind the left B-pillar. The Principal Direction Of Force (PDOF) was between 7:00 and 8:00 at a delta V of approximately 17mph.

The left rear door and structure behind the B-pillar deformed against the driver's seatback, keeping it upright. The female driver was uninjured but the male front seat passenger's seatback yielded rearward and left. He impacted the rear seatback between the two rear seat passengers and sustained a T8 spinal cord transaction with paraplegia. The two male rear seat passengers had minor injuries.

Case Three: A 1994 four-door sedan was stopped in a line of cars when it was hit from the rear by a mid-size foreign SUV. The sedan's air bag deployed when the car was pushed forward into the rear of a vehicle in front of it. Due to a faulty seat recliner mechanism, the seatback collapsed rearward. The male driver ramped rearward into the rear seatback, resulting in quadriplegia from fractures.

Seatback, Rigid, Yielding

C18 Rural Roads, Curves, and Vehicular Loss-of-Control

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After attending this presentation, attendees will obtain an understanding of the physical evidence that accompanies an event involving vehicular loss-of-control at a curve on a rural roadway. Original data will be presented on the chronological development of roadway edge drop-offs, the likelihood that travelling onto a drop-off will lead to a loss-of-control, the unusual motions of vehicles that might lead to a loss-of-control, the tire marks that identify a loss-of-control, and the actions drivers take when attempting to navigate through a challenging roadway curve.

This presentation will impact the forensic science community by providing orginal, objective data about the frequency of loss-of-control evidence on rural roadways that is often not officially reported, but the larger-than-reported frequency of these events may provide more accurate information about how and why loss-of-control events occur.

The loss of directional control of a vehicle leads to single vehicle rollovers and impacts with trees, poles, and roadside obstacles. It is also present prior to many serious and fatal head-on collisions. It has been observed that a large number of these events occur at curves of rural, two-way, two-lane roadways. Objective data is needed to understand how and why these events occur.

This presentation will provide a review of the physical evidence of vehicle loss-of-control that existed over three years (2009 – 2012) at the site of a complex S-curve in rural southern Ontario, Canada. This S-curve is made up of one segment that contains a small horizontal radius and a second segment of a larger radius. The segment with the shorter radius also contains an undesirable vertical alignment within the horizontal curve that would appear to make travel aligned within the lane challenging.

Detailed video footage taken over six days has been examined to explore how vehicles progress through this curve. Observations of vehicles passing through the site are summarized to identify unusual motions that lead to the potential of loss-of-control. Those vehicles conducting unusual motions are documented with respect to their type/size and their speed and how they progressed through the curve in relation to a grid of markers that was set up to document their longitudinal and lateral position in their lane.

Evaluation of edge drop-offs and estimates of the number of vehicles exiting the paved road edge are provided. Historically, roadway edge drop-off has been considered a causal factor in many loss-of-control collisions, while debate has continued over what characteristics should require early repair. As municipalities, provinces, and states are pressured by budget deficits to minimize maintenance costs, the definition of what constitutes a reasonable level of public safety is shifted. New Minimum Maintenance Standards (MMS) in Ontario define that an edge drop-off of 8 centimeters (just over 3in) existing over a full distance of 20 meters (about 65ft) is the new threshold at which maintenance of the drop-off is required. This presentation will discuss data that was collected of the changes in the edge drop-off through the three-year study and will demonstrate that deep edge drop-offs over 11 centimeters (4.5in) existed at individual locations while the criterion of 8 centimeters along the total 20 meter distance was never met. Efforts to fill in the exposed edge drop-off by plowing and then shifting the gravel back toward the edge drop-off causes a shoulder surface of deep and soft gravel which is argued to be as unsafe as leaving the area untreated.

The location/type of loss-of-control evidence in 54 incidents is reviewed. A comparison is made between the location of these events and the features of the site to provide an estimate of which features are more likely to be associated with a vehicular loss-of-control. Characteristics of the loss-of-control tire marks resulting from these events are discussed so investigators can further their ability to detect and understand this evidence.

Documentation is provided from travels through the curve in an instrumented passenger car. Longitudinal and lateral accelerations are presented as well as driver actions (braking, steering) at select speeds. These data are compared to the location of the loss-of-control incidents.

Crash, Curve, Loss-of-Control

C19 Forensic Analysis of a Seat-Belted Occupant Ejection in a Rollover Collision

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The goal of this presentation is to illustrate to the forensic community that vehicle occupants wearing seat belts are still at risk of ejection and injury during a rollover collision.

This presentation will impact the forensic science community by illustrating the apparent risk of injury and/or ejection while wearing a seat belt in a rollover collision.

Introduction: Seat belts are the primary occupant restraint system in passenger cars, light trucks, and sport utility vehicles. In the event of a rollover collision, three-point lap and shoulder belts help to reduce the risk of injury due to impacts with interior components or ejection. However, it has been observed that complete ejection of seat-belted occupants can and does occur in rollover crashes.[1] This case study will detail the physical evidence supporting seat belt use at the time of collision and the subsequent forensic analysis of the occupant compartment as it relates to the ejected passenger.

Collision Sequence Overview: A 2001 model year SUV with driver and right front passenger was traveling on a highway with a posted speed limit of 70mph. A left rear tire tread belt separation caused vehicle oversteer characteristics and the SUV entered a clockwise yaw on the highway for approximately 160ft.[2] Upon entering the dirt shoulder, the vehicle continued to yaw for another 52ft before it

overturned, driver's side leading. The vehicle rolled approximately 147ft and came to rest on its wheels. The right front passenger was ejected during the rollover event.

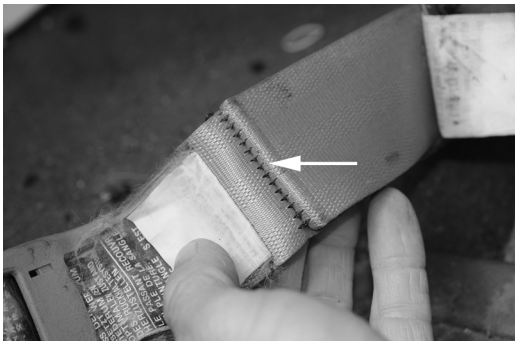
Based on stress marks observed on the driver's seat belt webbing, investigators concluded the driver was wearing the seat belt. The right front seat belt was found still buckled at the scene (Photograph 1), and the webbing did not reveal any type of stress marks. Although the investigators agreed the right front occupant was ejected, they concluded this occupant was either not wearing the seat belt properly, or was not wearing the seat belt at all.

Incident Vehicle Examination: Significant roof crush and corresponding occupant compartment intrusion is consistent with a driver's side leading rollover event (Photograph 2).[3] The left rear tire was examined and showed a near complete tread belt separation. Localized damage was observed around the left rear wheel well, on the rear bumper fascia, and exhaust system. Part of the tread belt was found still wrapped around the rear axle.

All of the vehicle's windows were fractured during the rollover. Careful examination of the right front seat belt showed approximately 15in of webbing with cut fibers consistent with interaction with fractured glass fragments. However, these fiber disturbances were found on an area of the webbing that would not be exposed to the vehicle's interior if the seat belt was stowed.

Artifacts aligned longitudinally with the webbing were found to correspond to faint striations on the plastic coating of the latch plate load surface.

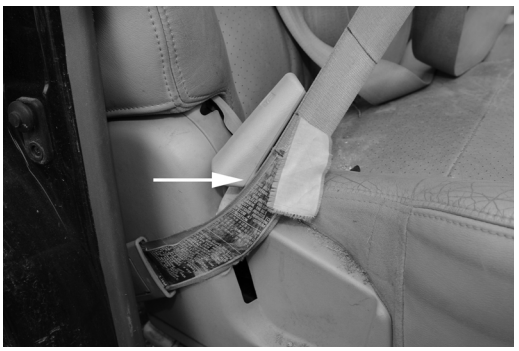
The right front seat belt features an expansion loop concealed under a rubber-like sleeve approximately 10in long.[4] Close examination of the first row of stitches revealed these stitches were under tension applied during occupant restraining forces (Photograph 3).



The right front seat back was found reclined beyond a reasonable upright posture such that the head restraint is in contact with the right roof rail within the right rear door opening. A courtesy light is installed on the interior upholstered right roof rail. The lens of the courtesy light exhibits linear scratches with plastic flow in an orientation directed toward the window and to the outside of the vehicle. The potential ejection portal was identified to be the right rear window opening.

Upon further examination, the seat back assembly was found to recline without much effort. That is, the seat back recline mechanism was not locked. The seat back recline angle adjuster is on the outboard side of the seat cushion. Pulling the lever upward allows the seat back to recline. This recline lever was found to be substantially deformed (Photograph 4).

When the right front seat belt is stowed, the webbing is nearly vertical, but when fastened the expansion loop sleeve rotates forward about the lower outboard anchor bolt. It was discovered that the rubber-like expansion loop sleeve will cause the potential for the seat belt webbing to route under the seat back recline lever (Photograph 5).



Exemplar Vehicle Examination: A similar model year exemplar vehicle was located by random selection. The right front seat cushion position of the incident vehicle was duplicated in the exemplar vehicle. When a right front occupant fastened the exemplar seat belt, the webbing caught on the tip of the recline lever. Remarkably, after the retractor was manually locked and the right front occupant rose up from the seat to put the lap belt in tension, the recline lever was actuated and the right front seat back unexpectedly reclined. The exemplar vehicle demonstration helped explain the chain of events that led to the seat-belted occupant ejection in a rollover collision.

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SUV Tread Separation, Rollover Ejection, Seat Belt

C20 Toyota® SUA: A Formal and Definitive Electronic SUA Examination Protocol

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The goal of this presentation is to show how the issue of the alleged Toyota® Sudden Unrequested Acceleration (SUA) has been the subject of media attention and intense Multi-District-Litigation (MDL). Various court protocols have been instituted in an attempt to standardize the examination of components, systems, and Event Data Recorder (EDR) event-records regarding the issue of the alleged SUA. This presentation teaches a method to amalgamate these protocols into a master procedure which specifies tests incorporating the three modes discussed above as possible for a specific Vehicle Under Test (VUT).

This presentation will impact the forensic science community by providing a tested and confirmed amalgamated master procedure that specifies tests incorporating the three alleged SUA vehicle test modes, such that the attendee will then be prepared for whatever field conditions he/she faces when encountering a vehicle subject to various court SUA protocols.

The issue of alleged Toyota® SUA has been the subject of media attention and intense MDL. Causes of alleged SUA can include mechanical, electrical, and computer control aspects. As a foundation to understand the technology involved, this presentation illustrates the architecture of the Toyota® Electronic Throttle Control systems (ETCS-i). This architecture includes an electronic accelerator function (Figure 1) and an electronic throttle function (Figure 2). The electronic accelerator provides a dual signal input to an Electronic Control Module (ECM) which represents an electrical accelerator-position-analog of the % pedal requested by the operator. The ECM acts on that signal, modulated by engine temperatures, current RPM, current vehicle speed, and other factors to provide a requested throttle angle to the electronic throttle. The electronic throttle actual angle is fed back via a dual signal Throttle Position Sensor (TPS), which produces an electronic throttle-position-analog to the ECM, thus forming a throttle closed-circuit feedback loop. Figure 3 is an overview schematic of this system. Additional to the primary throttle control system, the vehicle cruise control system is implemented within the ECM and it can also modulate the electronic throttle control.

In a crash event, the operating status of the vehicle is also monitored by an Event Data Recorder (EDR), and that is included in the protocol.

Examinations of these systems can happen in three modes:

1. Static component testing, no power to vehicle, including external EDR data retrieval.
2. Dynamic powered engine-not-running tests, including *in situ* EDR data retrieval.
3. Dynamic powered engine-running tests, including road tests

In Static Mode: the individual components are characterized individually by disconnecting them from the system to determine voltage and/or resistance outputs (throttle-analog and accelerator-analog) versus physical input or position. If power cannot be applied to the vehicle, the EDR data can be retrieved in a stand-alone mode.

In Dynamic Powered Engine-Not-Running Mode: the super system (accelerator, ECM, throttle) is monitored at its interface points and then actuated electrically to characterize the running voltages at the interfaces of all these components in a dynamic sense. This includes simultaneous time line recordation of CAN bus PID parameters, analog voltage parameters, and physical component positions (via synchronized four-channel video). One part of these tests is known as a "throttle-sweep," where the accelerator is actuated in fast, slow, and pulse modes to check for the compliant operation of the throttle vs. accelerator, while monitoring the above parameters. With power applied to the vehicle, the EDR data can be usually be retrieved via the normal SAE J1962 diagnostic port.

Figure 4 is an overview schematic detailing the interfaces required to accomplish the engine controls master procedure monitor points. Note that in order to accommodate a wide variety of vehicle models, the protocol includes the use of Insulation Displacing Connectors (IDC) which allow adaption to each vehicle version of interest.

In Dynamic Powered Engine-Running Tests, Including Road Tests Mode: the super system (accelerator, ECM, throttle) is monitored as above, but additional CAN bus parameters are recorded (Engine RPM, vehicle speed, brake apply functions, coolant temperature, etc.). Before such tests, it is assumed that all EDR data has been retrieved.

Generally, serious case vehicles are operationally damaged, and various court protocols have been instituted in an attempt to standardize the examination of ETCS-i components and systems, and EDR event-records on these vehicles. Thus, the application of protocol modes is vehicle condition-dependent. This presentation shows key portions of these protocols and then provides an amalgamation of these protocols into a Toyota® SUA investigation master procedure which provides for tests incorporating the three modes discussed above as possible for a specific VUT.

One of the key aspects of an alleged SUA investigation is the retrieval and interpretation of EDR data. As is commonly understood, the EDR function is incorporated into the SRS ECU. Aside from recording internal signal parameters (acceleration, etc.), the SRS ECU acquires PID parameters (braking, vehicle speed, engine RPM, throttle angle, etc.) from the CAN bus and that is the basis of an "event record." The correct/incorrect operation of the ETCS-i system is one of the keys to assessing the reliability of the EDR data.

So, the application of the ECTS-i portion of the master procedure can serve to confirm or cast doubt upon the EDR recorded event-record.

An SUA investigation, using this master procedure, is illustrated with examples of simultaneous timeline observation and documentation of analog parameters, CAN PID parameters, and multi-channel video recordation of mechanical operations versus those electrical parameters. Additionally, this presentation includes illustrations and discussion of EDR data, control pedal measurements, ECM data, ABS data, and ESC data.

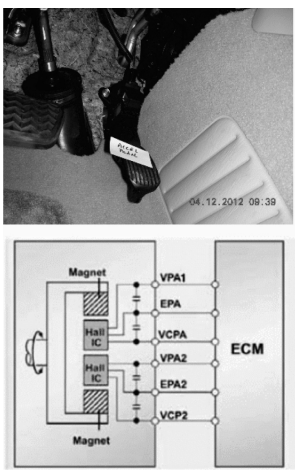

Figure 1. Electronic accelerator

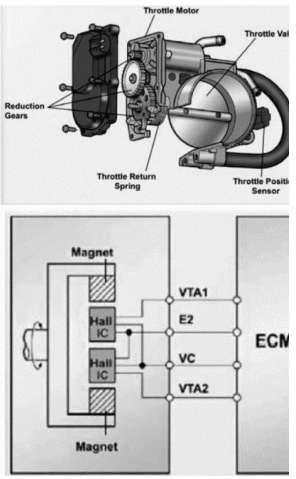

Figure 2. Electronic throttle.

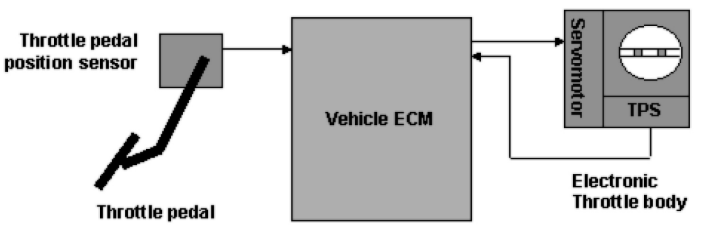

Figure 3. System schematic.

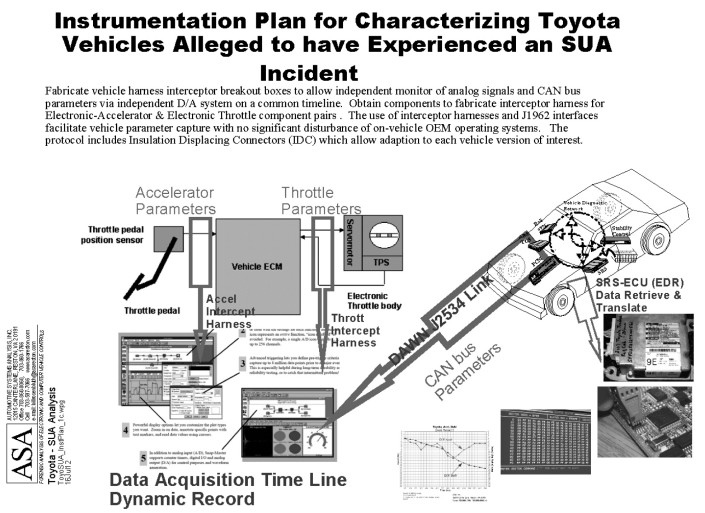

Figure 4. Overview system schematic.

Toyota® SUA, Toyota® MDL, Sudden Acceleration

C21 Evaluation of Roof-Mounted Side Curtain Airbags in Rollover Accidents

Michelle R. Hoffman, MS, Carley C. Ward, PhD, and Matthew A. Ivory, BS, 3720 E LaSalle St, Phoenix, AZ 85040*

After attending this presentation, attendees will understand how side curtain airbags protect occupants from head injury in side impact and rollover crashes.

This presentation will impact the forensic science community by explaining how roof-mounted side curtain airbags work and how to identify situations in which they have not performed in their intended manner.

History of Side Curtain Airbags: Roof-mounted inflatable side curtain airbags are designed to protect an occupant from ejection through a side window and prevent injurious head contacts to interior and exterior surfaces. They first appeared in passenger vehicles in the late 1990s.[1] They were used primarily for side impact protection.

The National Highway Traffic Safety Administration (NHTSA) tested vehicles in side impacts using a moving deformable barrier modeled after a passenger car of the 1970s. This test only examined forces on the crash test dummy's thorax and ignored forces on the dummy's head. In the early 2000s, the Insurance Institute for Highway Safety (IIHS) developed a side impact test that utilized a moving barrier that was higher than the one used in the NHTSA tests. This higher barrier was representative of a pickup or SUV. The new barrier made it possible for the crash test dummy to hit the striking barrier through the side window. In addition, the IIHS test examined the forces exerted on the dummy's head. It was felt that this new test would encourage the automakers to take advantage of the new technology of side impact head airbags. This technology was not in existence at the time that the NHTSA had created their side impact test.[2,3] The NHTSA followed IIHS's example and modified their side impact test to encourage the use of side curtain airbags. In the final rule on side impact protection (FMVSS 214), the NHTSA changed the side impact test requirements to include a lateral vehicle-into-pole impact in which the pole was positioned in line with the driver dummy's head.[4]

Side curtain airbags have also been improved upon to offer protection to occupants in the event of a rollover crash. In 2000, a major automaker offered an SUV with a side curtain airbag option that would deploy in rollover crashes. The side curtain airbags would trigger by a sensor that measured vehicle tilt and the bags would remain inflated for six seconds covering the side windows.[5] Since that time, many automakers have produced vehicles with side curtain airbags large enough to cover window openings and that remain inflated long enough to offer protection in a rollover-type crash. Recently, NHTSA's final rule on ejection mitigation (FMVSS 226) required all vehicles by September 2013 to be equipped with a curtain airbag that will deploy and offer protection in both side impacts and rollovers.[6]

Case Studies: To illustrate how side curtain airbags may fail to deploy in their intended manner, two cases are reviewed.

In the first case, the restrained left rear occupant of an SUV sustained fatal head injuries in a lateral impact and rollover crash. The side curtain airbags in the SUV did not deploy, allowing the occupant to become completely ejected. The question arose whether or not the side curtain airbag would have stayed inflated long enough to prevent the occupant's ejection and injury if it had deployed. Testing was conducted on exemplar side curtain airbags to determine the deployment path, size of the airbags, and time the bags would remain inflated. It was found that the side curtain airbag would remain inflated with enough pressure for a rollover event.

In the second case, the restrained right front passenger sustained fatal head injuries when she was partially ejected during a vehicle rollover crash. The vehicle was equipped with side curtain airbags that deployed but got stuck halfway down. A review of the NHTSA side crash tests demonstrated that this same problem had also occurred in New Car Assessment Program (NCAP) testing. Subsequent NHTSA NCAP side impact testing revealed that a design change had occurred that prevented this problem from occurring in later models. The issue in this case was what changes had been made to the side curtain airbags to eliminate the problem and would it have made a difference in the subject accident. Exemplar airbags from the early production and late production vehicles were obtained and examined. The later production side curtain airbags were changed by the addition of a plastic guard to prevent the airbag from snagging on interior components during deployment. This guard would have allowed the airbag to fully deploy in the subject accident.

Conclusions: Side curtain airbags are designed to protect an occupant from ejection through a side window and prevent injurious head contacts. They are quickly becoming an important part of automotive safety. An individual analysis must be performed to determine if they functioned properly.

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Side Curtain Airbags, Side Impact, Rollover

C22 Relationship Between Injury Severity and Vehicle Crush in Rear Impact Collisions

John J. Smith, MSEE, PE, Torey Jones, and Devin Jones, 43766 Buckskin Rd, Parker, CO 80138*

After attending this presentation, attendees will have a greater understanding of the relationship between crush and injuries in collisions.

This presentation will impact the forensic science community by enabling attendees to more accurately analyze injury mechanisms in rear impact collisions.

The objective of the research reported in this paper was to determine if the magnitude of crush to the rear of a vehicle was indicative of the severity of the collision. The research revealed that a statistically significant correlation between the crush to the vehicle and the Abbreviated Injury Scale (AIS) injury rating was not present. This confirms that injury severity is a function of more than the crush to the vehicle and the change in velocity.

It is well known that the energy available to cause injury is a function of the non-dissipated kinetic energy in a collision. Previously published papers have raised doubts regarding the correlation of the change of velocity experienced by a vehicle in rear impact and the resultant injuries.[1,2] Similarly, research has shown a lack of an established injury threshold based on change of velocity.[1,3-9] Theoretically, the greater the damage to the vehicle, the less energy is available to cause injury at a given speed. However, the energy available to crush a vehicle increases with the square of the velocity while the momentum is a linear function. Previous research has demonstrated an energy threshold value for the onset of damage in a rear impact.[9] This is relevant because at the lower speeds momentum is the critical factor while energy is the critical element at higher speeds.

The National Automotive Sampling System (NASS) is a database of more 100,000 collisions investigated by the National Highway Traffic Safety Administration (NHTSA).[10] The various versions of the database cover well over a decade. The AIS is a measure of the severity of an injury based on lethality.[11] The NASS databases record the injuries to an occupant in terms of type, cause, and severity. The files also report crush measurements, collision sequences, and change in velocity. Two significant limitations of the database deal with the change in velocity and the severity of the injury. Velocities are often calculated using SMASH, a derivative program of CRASH3 which has limited utility in rear impacts.[12] The injuries are based primarily on initial diagnosis and do not capture subsequent diagnoses which often raise the overall severity of the injury. As an example, injuries originally coded as AIS 1 may in reality be AIS 2 or 3. However, the crush to a vehicle is actually physically measured by the investigator. For these reasons, an analysis was performed to determine if the crush to the rear of a vehicle is statistically significant in the prevalence of diagnosed injuries in rear impacts.

The crush to vehicles and the maximum AIS code for a given collision were retrieved by examining a random sample of 198 impacts taken from the NASS case list. In an attempt to obtain an unbiased sample, every 20th case in the database was selected. To be chosen, the collision had to meet certain qualifications. Only rear impacts based on an impact angle of 180° were allowed. In addition, the collision had to be a pure rear end crash with no other sources of damage such as rollover or subsequent contact with other objects. The impact could only involve two vehicles. In addition, the database had to include the injury and AIS code and include the measured crush sustained by the vehicle. If any of these conditions were not met, that case was skipped and the next case was used.

Table 1 provides the results obtained by the research. The table compares the maximum AIS with the magnitude of the measured crush in inches. The number in the table lists the number of collisions in a given range. Figure 1 is a graph of the data with greater crush discrimination. Figure 1 also includes a linear and a quadratic fit to the data.

AIS	0 < crush < 10	10 ≤ crush < 20	20 ≤ crush < 30	30 ≤ crush < 40	40 ≤ crush < 52
1	72	56	32	8	3
2	3	8	2	1	0
3	0	1	1	2	1
4	0	0	0	0	2
5	1	0	2	1	0
6	0	0	0	0	2

Table 1 – Distribution of AIS based on inches of crush

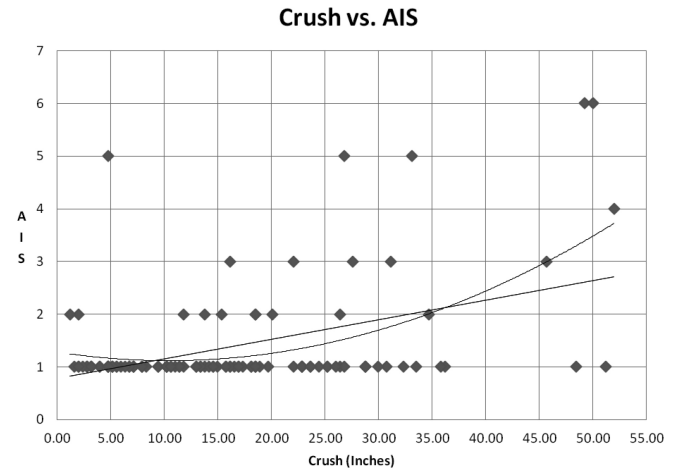

Figure 1 – Crush vs. AIS
Note: Many of the points are duplicates

An analysis was performed to determine if there was a statistically relevant correlation between the amount of crush and the initial injury level. Three tests were run on the data; Chi Squared, correlation, and Student-t. The latter was used to compare individual AIS levels. Additionally, as mentioned previously, a linear and quadratic fit to the data was determined.

Conclusion: The data reviewed demonstrates that for AIS level 1 and 2 injuries, crush to the vehicle is not a reliable indicator of injury potential. Additionally, severe injuries can occur with minimal crush while minor injuries can occur with significant crush.

None of the analysis methods applied to the complete data set resulted in a finding of statistical significance between crush depth and injury severity. The mean crush for each level was determined. A student-t test was applied to each AIS level and resulted in a finding of a statistically significant variation between AIS 1 and 2 versus AIS 3. However, it should be noted that of the 198 random sample cases, only thirteen revealed injury severities of AIS 3 or greater. The data does indicate that it is possible crush is a factor in AIS level 3 and higher injuries.

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Injury, Crush, Damage

C23 Performance Evaluation of Firearm Silencers

John Nixon, MBA, ARC, PO Box 66, Bippus, IN 46713*

After attending this presentation, attendees will gain knowledge of what a firearm silencer is, how it is constructed, the safety and environmental advantages of silencers, and how silencer performance is evaluated. It is anticipated that the presentation will be of particular interest to medical professionals and criminal defense attorneys—particularly those who work in the federal arena.

This presentation will impact the forensic science community by changing the way firearm silencers are viewed, dispelling common misconceptions, and promoting research into evaluation techniques, plus health, environmental, and legal issues.

Firearms use comes with a number of potential health and environmental hazards due to the high noise levels generated. Clearly, noise reduction is a desirable goal. Firearm silencers are often more correctly referred to as sound suppressors, sound moderators, or mufflers—in the interests of consistency with federal legislation, the term "silencer" is used throughout this presentation. Firearm silencers are legal for civilian ownership and use in the majority of the U.S., though they are heavily regulated. In states where silencers are legal, their regulation is typically accomplished at the federal level, though many states do have their own laws. This presentation focuses on issues pertinent to federal legislation.

Until 1934, there were no restrictions on silencer ownership and use by private individuals. In 1934, Congress passed the National Firearms Act (NFA) which effectively made silencer manufacture and possession by private citizens, a prohibitively costly and administratively burdensome endeavor.

The NFA legislation is articulated in Title 26 of the United States Code, and this federal law is administered and enforced by the U.S. Department of Justice (DOJ) via the Bureau of Alcohol, Tobacco, Firearms and Explosives (BATFE).

The U.S. Code defines, in part, a silencer or muffler, as "any device for silencing, muffling, or diminishing the report of a portable firearm." This is a very vague definition, and in strict technical terms, it could include legitimate technologies, such as a lengthened barrel, a muzzle brake, or a flash hider, all of which will diminish, or redirect, the noise of a gunshot.

Commercially available silencers reduce firearm peak sound pressure by 30 to 40 decibels (dB). A 223 Remington (5.56mm NATO) caliber rifle will typically generate around 160dB without a silencer and just 120dB to 130dB with a typical commercially available silencer installed. Some individuals construct homemade silencers, with varying degrees of success.

Understanding sound and sound measurement is not easy, and this fact, coupled with the loose legal definition of what constitutes a silencer, leads to the prosecution of many individuals who, either deliberately or inadvertently, have procured or created a device for their firearm that reduces the measured report by only a small amount.

A study published by the American Medical Association revealed that recreational shooters suffered hearing damage following only very limited exposure to firearms noise and that damage occurred even when shooters wore hearing protection. Paradoxically, in an age when both legislators and the population at large are obsessed with environmental and health and safety issues, there exists outdated legislation in place that actually works to make an everyday item less safe for the user and less environmentally friendly from the perspective of those in the vicinity of the user.

Sound measuring equipment with a data sample time interval short enough to accurately capture a gunshot is expensive, and interpretation of results is difficult for those not well versed in the technical intricacies of acoustics.

This presentation includes a case study to illustrate the difficulty in applying the U.S. Code relating to silencers in a criminal prosecution.

It is concluded that current legislation relating to silencers provides vague technical definitions, leads to unnecessary and expensive prosecutions, has a significant adverse effect on the health of the nation, and raises significant legal, administrative, and financial barriers to those firearm owners who wish to maximize safety and minimize environmental impact.

Firearm Silencer Evaluation, Hearing Damage, Environmental Impact

C24 Was That Car Used as a Weapon or Not? Combining Reconstruction Skills to Answer a Critical Question

Michael L. Shirley, BSME, PE, 2290 County Rd 23, Waterloo, IN 46793-9415; and John Nixon, MBA*, ARC, PO Box 66, Bippus, IN 46713*

After attending this presentation, attendees will gain knowledge in the mechanics of shooting incident reconstruction and vehicle incident reconstruction, and how the two disciplines complemented one another in providing a definitive answer to a key question that was critical to an investigation and the outcome of an associated civil lawsuit. It is anticipated that the presentation will be of particular interest to engineers and that it will be of benefit to both civil and criminal attorneys and their investigators.

This presentation will impact the forensic science community by discussing how incidents involving firearms and automobiles are investigated by highlighting the overlapping technologies and demonstrating how the two disciplines complement one another when the experts work as a team.

The presentation focuses on a real world practical example of a life-and-death struggle that developed from a routine traffic stop. When a law enforcement officer pulled over a lone driver following a minor traffic infraction on a suburban roadway, he did not expect a major struggle. However, after providing false identification, the suspect became aggressive and combative and the officer called for backup. The suspect was extremely strong and the two officers used Electronic Control Devices (ECDs) in a futile attempt to subdue him. The struggle lasted for several minutes and the suspect eventually managed to get behind the wheel of his manual transmission car and move off at full throttle. One of the officers was in the vicinity of the front driver's side of the vehicle and, fearing for his safety, both officers fired shots from their handguns. Bullets entered the vehicle and the suspect was fatally wounded.

Attorneys, acting on behalf of the suspect's estate, filed a lawsuit against the two officers and their employer. The plaintiff's attorney hired a shooting reconstruction expert who produced a report that indicated the police officer at the front of the car was two to four feet away from the vehicle and, consequently, was not in danger at the time the officers fired their guns and killed the suspect. The plaintiff's expert report made a number of assumptions with regard to the incident and, importantly, the lone expert had very limited technical knowledge of vehicle mechanics and dynamics.

The defense experts conducted an investigation to determine if the police officer at the front of the vehicle was in any danger at the time the officers opened fire. The investigation began by using trajectory analysis to determine the trajectory of bullets that entered the vehicle through the front windshield. Once this was ascertained, the officer was instructed

to adopt his shooting stance and he was positioned so that his pistol was coincident with the trajectory line of the bullet going through the windshield. This established the position of the officer to be approximately 16" forward of the driver's side front wheel of the suspect's car.

The next step of the investigation was to determine if the officer was in any danger while standing in this position. Witness testimony stated that the suspect applied full left lock as he pulled away in his car at full throttle. The vehicle was examined and the steering geometry measured. Calculations were performed to determine the turn radius of the vehicle at full lock. Industry test data was used to calculate the acceleration of the vehicle as it sped away from a standing start. The turn radius, vehicle acceleration data, and the location of the police officer were used to perform calculations. The calculations demonstrated that it would have taken less than 0.5 seconds for the suspect's car to reach the officer. Additionally, a practical demonstration was performed with the officer and the vehicle—it demonstrated that the driver's side front wheel of the vehicle would have run over the police officer.

The combined use of measurements, calculations, and practical demonstration was sufficient to prove that the police officer was in danger at the time of the shooting, and that the shooting was justified. The combined expertise of two engineering disciplines proved invaluable in this investigation.

Bullet Trajectory, Vehicle Engineering, Incident Reconstruction

C25 Who Pulled the Trigger? Blood Evidence and Bullet Path Determine Whether Shooter Was Friend or Foe

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The goal of this presentation is to demonstrate how basic physics principles were used to determine who shot a man in the neck. An analysis of the blood evidence and the bullet path was used to identify who pulled the trigger.

This presentation will impact the forensic science community by showing a unique case where both the body motions of the individual who was shot and the motion of the vehicle in which he was a passenger were factors used in determining where the shooter was physically located and, ultimately, who fired the gun.

Summary of Case Facts: An adult male was the right front passenger in a sedan when he sustained gunshot wounds to his neck and right shoulder.

The sedan's driver told police that he and the victim were sitting in the vehicle parked near the corner of their apartment complex when three males approached the vehicle. The driver backed up to get away, and when he was halfway out of the parking spot, he heard three shots coming from the right rear window, which was rolled halfway down. He continued backing, hit a curb, drove forward and stopped directly in front of their apartment. The victim's wife came out and observed that her husband was bleeding from the right side of his neck. She and the driver then moved the victim to her vehicle, whereupon she took him to the hospital, which reported the incident to police.

When police inspected the vehicle, the right rear window was partially down. Blood was on the front passenger seat. Drips of blood were on the passenger B-pillar trim and doorframe of the right rear door. A .380 semi-automatic spent casing was on the back seat. No casings were found outside the vehicle at the scene. There were no slugs inside the vehicle or evidence of bullet strikes.

The victim's wife later told police she believed the driver shot her husband. She did not see anyone or any vehicles leave the scene. She stated that the driver had threatened to kill her husband a month earlier when they got into a fight.

A family friend also told police she suspected the driver and suggested he and the wife were lovers, and may have colluded to commit the assault.

Injuries: The victim sustained three external gunshot wounds to his right shoulder and neck and spinal fracture at C2. A bullet remained in the left lateral soft tissues of the neck. He had right upper extremity paralysis and right lower extremity weakness.

Analysis: Only one casing was ever found at the scene and it was in plain view on the sedan's back seat. The single bullet was lodged in the victim's neck. With the victim's right shoulder shrugged up toward his right ear, one bullet could have caused all of his wounds. The bullet entered the right shoulder just above the clavicle and exited just medial and forward of the entry location before it re-entered his body on the right side of his neck at C2. The bullet shattered C2 and lodged in the soft tissues on the left side of his neck.

The bullet contact locations were reproduced on a surrogate victim in an exemplar vehicle for two scenarios: (1) the gun being fired through the vehicle's right rear window with the victim seated in the right front seat; and, (2) the gun being fired by the driver as the victim exited the vehicle. A rod was used to analyze the bullet path in these scenarios. Both scenarios were possible, but it was unlikely that one could fire through the rear window while the vehicle was backing up. Also, the casing location was consistent with the scenario of the driver firing the gun.

Blood evidence was also more consistent with the second scenario. Blood droplets traveled vertically downward along the B-pillar trim before traveling at an angle toward the front of the car. Tests were conducted that showed that blood would travel down the B-pillar due to gravity, but would travel at an angle similar to that in the car due to rapid vehicle backing. This indicated the blood on the inside of the vehicle traveled vertically from gravity before the vehicle backed up. Significantly, this is inconsistent with the driver's statement that shots were fired as he was backing the car.

Conclusions: Due to circumstances at the time of the crime, evidence was lost and no one was prosecuted. However, later in a civil suit, the available evidence showed the driver shot his passenger.

Shooting, Blood Evidence, Bullet Path

C26 Fatal Injuries From Penetration by Weapons — Thrown or Stabbed?

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The goals of this presentation are to consider the defense that is commonly used in fatal cases of penetration by implements such as knives, screwdrivers, or scissors, that the implement was thrown to create the injury, as opposed to stabbed, and to discuss the distances over which the weapon is claimed to have been thrown, which is typically several meters. The questions that will be addressed are: (1) is it possible for implements such as knives, screwdrivers, or scissors to penetrate human tissues to sufficient depth to create fatal injuries by a throw?; (2) how much energy is required for penetration of skin and the underlying tissues, and at what speed would the implement have to be thrown?; and, (3) can the implements be thrown with sufficient accuracy to make penetration by the blades likely or does the yaw, pitch, and tumble prevent the implements from penetrating?

This presentation will impact the forensic science community, particularly forensic pathologists but also forensic scientists and engineers who are asked to provide expert opinions on the likelihood of a stabbing versus throwing in fatal cases, by discussing whether implements used in causing death from penetrative injuries can be thrown to give the same injuries as a stab wound.

Kitchen knives, screwdrivers, and scissors are not designed for throwing. When they are thrown, there tends to be considerable yaw, pitch, roll, and tumble during flight.

It is difficult to throw an implement with precision such that it orientates itself with the point foremost on impact. Even over short distances, the rotation from throwing the implement will often lead to it impacting handle first.

Whether or not an implement would have sufficient energy to penetrate the body can be estimated by calculating the kinetic energy.

The kinetic energy of an implement can be calculated from the formula K.E. = $0.5mv^2$ where m is the mass of the knife and v is the throwing velocity. The mass of the implement and maximum throwing velocity then need to be known for someone throwing that implement.

O'Callaghan *et al.* have previously considered the throwing of a glass shard and found that the maximum throwing velocity that could be obtained was $20ms^{-1}$.[1] They measured the energy required to penetrate skin and found for a sharp glass shard an energy of 7.7 J was required to allow skin penetration.

Figure 1 shows calculation of the relationship between throwing velocity and impact energy for a typical weapon. The threshold throwing velocity to allow skin impact is arrowed.

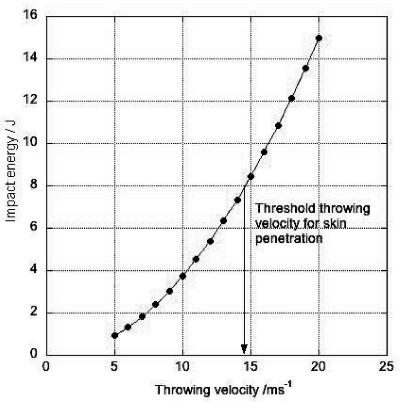

Figure 1: Impact energy versus throwing velocity for a 75g weapon. Assuming a sharp tip and that skin penetration occurs at 7.7 J, the threshold throwing velocity for skin penetration is $14.5ms^{-1}$.

This presentation will show the range of throwing velocities that can be achieved by throwers with knives, screwdrivers, and scissors and the ability of thrown knives to penetrate skin. The energy for skin penetration will be measured for the various implements and compared to their sharpness. The presentation will illustrate the different throwing characteristics of the different implements and discuss the implications of the results in interpreting throwing versus stabbing scenarios.

Reference:

1. P.T. O'Callaghan, M.D. Jones, D.S. James, S. Leadbeatter, S.L. Evans, L.D.M. Nokes, A biomechanical reconstruction of a wound caused by a glass shard—a case report, Forensic Science International 117 (2001) 221-231

Stabbing, Throwing, Sharpness

C27 A Forensic Investigation Methodology for Accidental Events in Process Plants

Alberto L. Geraci, PhD, Via Domenico Tempio 3, Acicastello, Catania 95021, ITALY*

The goal of this presentation is to propose an investigative methodology to be used in forensics for accidental events occurring in process plants, to help judges determine the truth.

This presentation will impact the forensic science community by showing how the proposed methodology is based on the experience gained over the years in investigating processing plant accidents. It includes a number of cases where the accident was reconstructed and the causes were identified. Additionally, suggestions are made to prevent these accidents from happening again. Finally, ways to establish accident responsibility are shown.

Often because of the condition of aging equipment, existing plants are at risk of accidents. Even if maintenance procedures are fully compliant with required rules (API, ASME, ASTM), company standards, and local by-laws, there is still a risk of a defect. A crack may generate leakage, which is a danger for fire and explosion. Bad operations, lack of procedures, maintenance carelessness, negligence, and human errors are among the causes of a processing plant accident. According to statistics of a large number of pipe leakage accidents, media corrosion and cavitation erosion are the main reasons leading to accidental events. The percentage is higher than 50% of the total accidents.

A plant accident, often with deaths, usually leads to a court case with a judge asking why the accidental event occurred and if it should have been avoided, in order to assign responsibility. The proposed investigation methodology is based on the experience gained as a judge's consultant at different courts of law in Sicily, Italy, close to processing plant sites, and experience as a professor and researcher at the University of Catania in Sicily, working in the same field. The methodology deals with a routine procedure to be applied to process plant accident court cases.

Starting from testimonial information acquired after an accident, plant documentation is acquired and studied carefully. It includes drawings (plot plans, layouts, flow charts, Piping And Instruments Diagrams (P&ID)), operative manuals, equipment materials, and maintenance programs. Compliance with codes and standards, in force for that type of plant and the related country, is verified. Different rules are compared and checked to determine if they were followed carefully, as required. Plant components affected by the accident are tested first with Non-Destructive Testing (NDT) methods selected for the suitable application to the case.

Among them, an important role is played by Thermal Infrared Imagery (TIR), often coupled with another suitable NDT method for on-stream defects detection. This technique, developed in this research capacity, was named "adapted thermography." Subsequently, the components are subjected to destructive tests (not repeatable), to check how the accident occurred and its causes. The above tests are preceded by recalls of the main theory fundamentals, making the proposed methodology more scientifically founded.

When the causes of the accident are found and the event is reconstructed, organization charts of the personnel involved are acquired, to determine responsibility and prosecution. Several study cases regarding accidental events which occurred at the petrochemical sites of Priolo and Gela (both in Sicily), are reported and discussed with a number of figures, tables, photographs, and diagrams. The investigation was directly carried out, constituting a first-hand application of the proposed investigation methodology.

There is general expectation the methodology will become a significant part of the forensic activity of involved academic professors, researchers, engineers and professionals in the field, and judicial personnel (lawyers, judges, consultants). In fact, it provides a new routine investigation tool, for court cases involving process plant accidents.

Plant Accidents, Mechanical, Investigation

C28 A Near Electrocution by Irrigation Pipe — Case Solved by Photo Inspection

Adam K. Aleksander, PhD, Aleksander & Associates, PA, PO Box 140558, Boise, ID 83714*

After attending this presentation, attendees will better appreciate how reviewing previously produced evidence can be very rewarding and can decide the case.

This presentation will impact the forensic science community by demonstrating how a false theory can be defeated by careful examination of the evidence.

This case that resulted in litigation occurred in the large agricultural fields of eastern Idaho. A field worker reported he had been burned and

almost electrocuted while touching or moving an irrigation pipe he found on the ground during a field clearing operation. The worker was well experienced and presented a story that at first seemed plausible; however, as the utility company and their experts reviewed the circumstances of the case, it became clear that the story represented by the worker simply did not add up. Further complicating the issue was the report by an electrical engineering expert witness who presented theories that were novel at best and unsupported by the evidence at worst. This plaintiff's expert's testimony, late in the case, meant that a jury might hear only his testimony, and no opposing view, as most of the investigators were associated in some way with the utility company.

The task of this defense investigator was to enter the case very late in the process and try to determine what happened, why, and how the plaintiff's theories were defective. The investigation combined elements of electrical engineering, human factors engineering, safety engineering, and a review of all utility records, investigation photos from the utility and sheriff's departments, as well as medical and other records.

This presentation will outline the evidence in the case, using photographic records from the various investigating agencies as well as original photos, to set the scene.

A systematic review of the evidence, including burn marks on cables, irrigation pipe, on the worker's hands, feet, and head, as well as other demonstrative evidence, will demonstrate the challenge facing the defendants.

At the heart of the matter were theories that, however illogical to the trained expert, were nevertheless compelling enough that a rural jury might be sufficiently swayed to make the verdict uncertain. These theories ignored the routine practice of utility companies to record and time stamp each event that might involve a circuit trip or fault. A detailed millisecond record existed of the initial trip event, an automatic re-set and final trip, and circuit disconnect.

In a review of routine photos that were thought to be of little significance, the missing link was revealed that finally answered the key defense question: why did the field worker raise the pipe into the 9kv electrified line, and why was his story that he found the pipe on the ground not plausible?

Part of the analysis was the investigation of similar incidents in the agricultural field, where contact with an overhead power line is unfortunately not uncommon. There is a significant statistical body of information that identifies contact by irrigation pipes and overhead lines as a major safety concern. It is this investigation into the common practice by field handlers of irrigation pipes that linked with the photographic evidence, finally providing a plausible explanation for the case.

A few comments will be made about a very short and suddenly abbreviated deposition as well as the surprise ending at trial.

Irrigation, Electrocution, Voltage

C29 Safety Evaluation of Post-Manufacture Firearm Modifications

John Nixon, MBA, ARC, PO Box 66, Bippus, IN 46713*

After attending this presentation, attendees will gain knowledge in the mechanics of firearms, how they may be modified after leaving the manufacturer, and how practical, instrumented, and calculative techniques may be employed to assess those modifications from the perspective of both performance and safety. It is anticipated that the presentation will be of particular interest to engineers, and that it will be of benefit to both civil and criminal attorneys and their investigators.

This presentation will impact the forensic science community by discussing how firearms accidents, and claimed accidents, are investigated by proposing that the firearms be assessed using a holistic approach that combines traditional practical methods coupled with modern sophisticated instrumentation, and mathematical/engineering calculations.

The presentation focuses on a real world practical example of a rifle that was modified after leaving the manufacturer. The owner of the rifle felt that the trigger "feel" was not conducive to accurate shooting and had a local gunsmith do a "trigger job" on the rifle. Following modification, the rifle discharged inside a residence, causing serious injury to a third party. The shooter stated that the rifle discharged while he was attempting to unload it by first removing the magazine, and further stated that the rifle may have been bumped against a piece of furniture immediately prior to discharge. There was some question as to whether the rifle discharged accidentally due to mechanical defect or whether it may have been due to negligent or deliberate action on the part of the operator. Due to the severity of the injuries, criminal and civil investigations, and lawsuits, ensued.

No documentation or testimony was available from the gunsmith who performed the work on the rifle, and this meant the investigation needed to ascertain exactly how the trigger mechanism had been modified. This was achieved by comparison with an exemplar rifle—new and unmodified. The operation of the rifle is described, including its trigger mechanism and its safety mechanisms. The general trigger mechanism modification options available to gunsmiths are described, and their pros and cons discussed.

The evaluation procedure commenced with basic testing of the mechanical aspects of the trigger and safety systems in both the subject rifle and the exemplar rifle. This comprised a basic operation test, followed by shock and vibration testing. Next, the trigger mechanisms were tested using an electro-mechanical trigger test apparatus linked to a computer. Finally, the rifles were disassembled and the trigger mechanism components were visually and dimensionally examined and compared. This procedure included a full evaluation of the trigger-sear springs.

The test and evaluation data were used to perform some calculations with regard to operating forces within the trigger mechanism, and the implications of these results are discussed. It was concluded that the only modification to the trigger mechanism of the subject rifle was a replacement trigger-sear spring of unknown origin. A commercially available aftermarket trigger-sear spring was procured and installed in the exemplar rifle. The test procedures were repeated for that configuration and the results compared to the "as manufactured" exemplar and to the subject rifle. Finally, the safety implications for all three systems evaluated are discussed in the context of the incident under investigation, and for firearm safety in general.

Firearm Accident, Firearm Modifications, Trigger & Safety Analysis

C30 Toward a Dual-Host Approach for Sensing of Ammonium Nitrate and Other Explosive Salts: Intermolecular Interactions Between the Dansyl Fluorophore and Anions Lead to Large Fluorescence Enhancements

Amanda M. Howett, BSc, Lissette I. Lozano-Lewis, MSc, and Konstantinos Kavallieratos, PhD, Dept of Chemistry and Biochemistry, Florida Intl Univ, 11200 SW 8th St CP326, Miami, FL 33199-0001*

After attending the presentation, attendees will have a better understanding of the interactions between the dansyl fluorophore and anions such as NO_3-, Cl-, and Br-. Such intermolecular interactions lead to significant fluorescent intensity enhancements upon anion addition, and detailed studies of intermolecular interactions by fluorescence and NMR spectroscopic methods will be presented. This can lead to future design of novel sensing devices based on simple combinations of cation and anion hosts for selective detection of ion-pair explosives, such as ammonium nitrate and uronium nitrate.

This presentation will impact the forensic science community by introducing the principles and preliminary results of a simple dual-host extraction method for sensing ammonium nitrate and other salts. The key factor is the combination of cation- and anion-sensing elements for the detection of an ion pair via a dual-host combination. As an initial step, a detailed investigation of the interaction of the dansyl fluorophore with anions, such as nitrate, is presented. The short-term goal is a

thorough understanding of the remarkable fluorescence enhancements observed upon addition of anion solution to dansyl chloride, and manipulation of host structure to induce selectivity for nitrate, in particular. The longer term goal is to combine the anion-sensing moiety based on dansyl fluorophore with cationic hosts, selective for ammonium, and achieve selective sensing for specific ion pairs, such as ammonium nitrate.

Detection of explosives plays an important role in national security. After events such as the 1995 Oklahoma City Bombing, the Anti-Terrorism and Effective Death Penalty Act was passed in 1996. The Act requires that all explosives be tagged to assist in the discovery and tracking of explosive devices as well as banning untagged explosives; however, the problem of tagging explosive substances such as ammonium nitrate that are present in common, easily obtained items such as fertilizers, remains. Because of this issue of widespread ammonium nitrate availability, research has been, and is still being done, focusing on the detection of the ammonium nitrate specifically as an ion pair, and detection methods based on solvent extraction by a dual-host supramolecular approach have been proposed.

Analytical techniques that have been used in the past in the detection of ammonium nitrate include gas-liquid chromatography and ion chromatography. These techniques are useful; however, there were still areas for improvement. Gas-liquid chromatography has a limited detection of thermally liable explosives as well as low selectivity and sensitivity for nitrogen-containing compounds. The high temperatures used can easily decompose the thermally liable explosive. The weakness of ion chromatography is that of column depletion. As the column is used and becomes depleted, artifacts can be produced that interfere with the detection of the explosives.

Fluorescence detection presents a sensitive, inexpensive, and potentially portable method for in-the-field detection of explosives. Heteroditopic receptor systems based on dual-host binding and fluorescence offer the potential for unique sensing effects via binding and extraction to the organic phase of both ionic components of the explosive salt as a tight ion pair. The dual-host complex has two different receptors, one for each ion, specific to each of the ions. The low dielectric constant of the organic medium assures close contact of the two hosts, which is essential for unique signals upon ion pair binding as opposed to binding of only one of the components.

Preliminary experiments by fluorescence spectroscopy, NMR spectroscopy, IR, and ESI-MS offer some insight on the utility of the dansyl fluorophore for nitrate detection, via supramolecular interactions. Specifically, fluorescence titrations of a $1x10^{-5}$M dansyl chloride solution with tetrabutylammonium nitrate in CH_2Cl_2 showed dramatic fluorescence enhancements upon nitrate addition (Figure 1). The chemical shift changes observed at ^{1}H- NMR titrations (Figure 2), indicate fast exchange and are apparently due to supramolecular interactions (presumably anion-pi interactions) and not to a reaction of the dansyl chloride with any added species. Preliminary determination of the binding constant for the interaction of dansyl chloride with nitrate indicates a high number (in the vicinity of 10^5M^{-1}). It should be noted, however, that there was difficulty fitting the data into a simple 1:1 binding model, and the possibility of multiple equlibria or more complex models is currently being investigated. Experiments investigating the interactions of the dansyl moiety with other anions are also in progress.

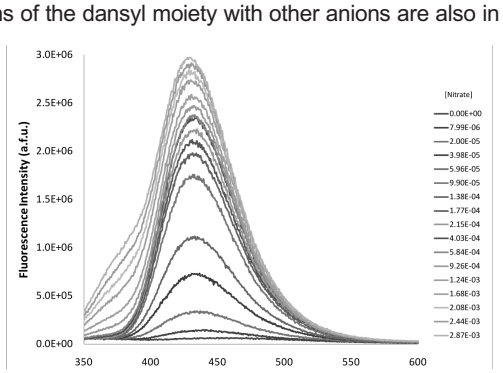

Figure 1: Fluorescence titration ($\lambda_{exc.}$. 310nm) of dansyl chloride (1 x 10^{-5}M) with Bu_4NNO_3

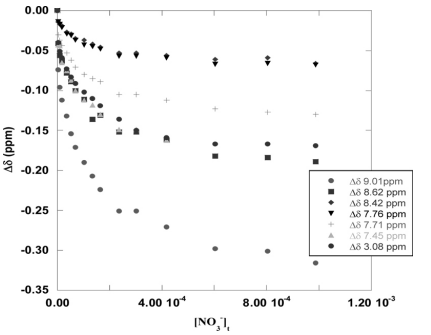

Figure 2: ^{1}H-NMR Chemical shift changes upon titration of dansyl chloride (5 x 10^{-4}M) with Bu_4NNO_3.

Fluorescence Sensing, Ammonium Nitrate, Dual-Host Extraction

C31 Principal Components Analysis Applied to the Gunshot Case Determination

Luis Chavez, BS, and Bladimir Espinoza, BS*, Anfion Muñoz 700, La Serena, Coquimbo, CHILE; and Cristian F. Lizama, BS*, Arturo Prat 19, Temuco, Araucania, CHILE*

After attending this presentation, attendees will understand the problem associated with determining who was the perpetrator of a criminal gunshot. The classical approach through the use of SEM-EXD is not possible for many developing countries due to the associated high costs. AA-GF equipment can be used as a replacement and the quantitative results interpreted with multivariate analysis techniques including Principal Components Analysis (PCA).

This presentation will impact the forensic science community by demonstrating the use of multivariate analysis giving forensic laboratories in non-developed countries the chance to determine with statistical certainty and with equipment of average value, the presence of gunshot residues on the hands of a suspect or the victim committing suicide. Through the construction of a solid database with several types of firearms, calibers, and ammunition trademarks, the quantitative analysis of metals present on the hands of an individual will allow its classification as “not shoot” and “shoot” situation, which graphically are visualized as clusters of data points represented in a 3D vector space. On the other hand, sampling is accomplished at a low cost with cotton swabs soaked with an EDTA solution along with the use of a universal reagent present in almost every chemical laboratory.

Forty volunteers with different jobs fired one different weapon each, including carbines, rifles, semiautomatic pistols, revolvers, shotguns, and submachine guns of the marks Mauser, Savage, italo Gra, Taurus, Smith and Wesson, and Sig Sauer, among others. Ammunition brands used included Remington, CBC, Federal, and TEC in the calibers .22LR, .38 special, 9mm Luger, 7x57mm, and the gauges 12 and 16. Samples were taken from the palm and back of the hands of the shooters with cotton swabs and 2% EDTA solution before and after every single shot. After dissolution and centrifugation, supernatants metal contents of lead, barium, and antimony were read using Atomic Absorption Spectrometry (AAS) into graphite furnace chambers. PCA was applied to the results, assuming that the following factors were analyzed: lead, barium, and antimony amount detected (each separately), shooter, zone analyzed in the hand and situation (before or after the gunshot). To simplify the analysis, all the variables were considered as numerical ones, defining before and after the shot as -1 and +1, identifying each shooter correlatively as 1, 2, 3, and so on. Zones tested on the hand were defined as -1 and +1 for back and palm, respectively.

Six eigenvalues were obtained for each of the sis principal components calculated previously. The first two values account for 68.9% of the variability of the data; the 3D eigenvalues explain at least 83.2% of the data variation. As could be expected, no correlation was observed between metal amount and the other variables. On the other hand, a strong relation between shooter and gunshot time-dependence is detected, which allows one to differentiate between after and before

the shot. When two score plots were displayed, at least three populations can be seen, which are split clearly into four populations, corresponding at before and after shot case for each zone on the hand.

Two cases of databases constructed for multivariated analysis of the results will be presented, showing in this way the universality of the procedure.

GSR, AA-GF, PCA

C32 Low-Energy Bone Fractures

David Pienkowski, PhD, Univ of Kentucky, Dept of Biomedical Engineering, Wenner Gren Laboratory, Lexington, KY 40506-0070*

The goals of this presentation are to increase awareness of low-energy bone fractures, review the factors potentially responsible for reducing fracture threshold, and facilitate reconciliation of low-energy events that will result in ostensibly disproportionate bone fractures.

This presentation will impact the forensic science community by reviewing the factors that reduce fracture thresholds and thereby assist the forensic investigator to reconcile seeming discrepancies between low-energy events and bone fractures. Forces calculated from reconstruction of low-velocity accidents or otherwise atraumatic incidents may appear incommensurately small compared to the accompanying human injuries. This can generate uncertainties regarding the reconstruction mechanics or incident events. Fractures of the appendicular skeleton accompanying low-energy incidents are becoming more prevalent and thus gaining greater attention.

The accident reconstructionist/human injury analyst quantifies event kinetics, calculates associated impact forces, and links these forces to the resulting injury by using probabilistic methods. Although the magnitudes of the event-related forces are generally proportional to the degree of injury, the relationship between force and injury in low-energy events is often nonlinear. Such disparities can lead to uncertainties regarding the validity of the reconstruction, inclusiveness of the events considered, or skill of the investigator.

These disparities may be partially resolved by considering the factors affecting bone's mechanical competence. Bone is both a calcium-ion reservoir and a dynamic mechanical load-bearing organ. These vital functions are enabled by a time-varying composite biomaterial with complex micro and macro skeletal structures. The mechanical competence of normal healthy bone is optimal; material or structural deviations from normal commonly result in compromised load-bearing capabilities and increased low-energy fracture susceptibility.

Bone material abnormalities can arise from inherited defects in bone metabolism that result in subnormal fracture resistance. Bone mass abnormalities are linked to sex hormone inadequacies (hypogonadism) or, more commonly, loss of estrogen in post-menopausal (natural or surgically-induced) women. Estrogen loss precedes the well-known (osteoporosis) rapid loss of bone mass due to supra-normal bone turnover. Antiresorptive drug therapies reduce turnover and bone loss, but new evidence suggests that long-term use of these drugs can adversely alter fracture resistance. This topic is currently under investigation due to the tens of millions with osteoporosis (growing ranks of aging baby boomers) and the widespread use of these drugs.

Lifestyle changes can also predispose bone to low-energy fracture. Bone mass increases rapidly during adolescence, peaks somewhere in the late-teens to mid-30s, then slowly declines throughout life. Evidence exists that exercise-induced bone mass increases persist to some degree throughout life, and conversely, inadequate skeletal loading during development prevents attainment of peak bone mass. Adults so affected begin "withdrawing bone" from a smaller "bone mass nest egg" and critical fracture thresholds are thus reached sooner.

Dietary insufficiencies also conspire against fracture resistance. Bone is a composite material made chiefly of protein and mineral (calcium hydroxyapatite). Current preferences for carbonated beverages instead of milk are thought responsible for deficiencies in calcium intake. Limiting sunlight exposure inhibits production of the active form of vitamin-D (essential for calcium absorption from the gut) and thus exacerbates the lack of calcium. Mild cases of vitamin-D deficiency are common even in otherwise well-nourished adults. Bone mass, critical for fracture resistance, cannot be gained or maintained in the absence of essential building materials.

Bone fractures are also appearing in response to low-energy events in young middle-aged pre-menopausal women with otherwise normal bone mass. The etiology of these fractures is unknown, but recent findings suggest structural defects in bone's protein component, notably abnormal collagen crosslinking.

Reconciliation of the force levels produced by low-energy events with resulting bone fractures requires a more thorough approach to understand this causal relationship. References linking force amplitudes with fractures offer data from populations, but understanding an individual's bone fracture threshold requires additional consideration of the particular material and structural aspects of the involved bone. This begins with a complete patient history, including prior surgeries, injuries, and medications. Non-invasive diagnostic tests quantifying the amount (bone mineral density) and current changes (biomarker assessments) in bone mass are also helpful and may exist in the subject's medical records. Bone biopsies are exceptionally useful, when available, and can provide the basis for a quantitative assessment of bone material composition and load-bearing structure. The forensic investigator should consider these and other factors to help understand the relationship between low-energy events, the accompanying forces, and the resulting bone injuries.

Human Injury, Injury Analyses, Accident Reconstruction

C33 Unusual Failure Modes of Large Diesel Generators in Bangladesh

Adam K. Aleksander, PhD, Aleksander & Associates, PA, PO Box 140558, Boise, ID 83714*

After attending this presentation, attendees will have a better appreciation of difficult operating environments for these very large engines, and the local challenges in a developing country such as Bangladesh. Furthermore, classic investigating techniques are still useful even in the face of extreme destruction.

This presentation will impact the forensic science community by emphasizing how conventional forensic engineering techniques still yield results even if the odds are poor.

Large diesel engines operating on compressed natural gas or heavy fuel oil are used in electrical generating stations. These engines operate at a nominal 500rpm, are turbocharged and intercooled, and can generate on the order of 17MW per engine.

One such installation utilizes eight engines and is located on a barge in Bangladesh, providing 118MW of power to the local grid. Due to local power dispatch requirements, these engines are often stopped and re-started as load demand fluctuates. These engines accumulate many hours and many start-stop cycles. This project had accumulated some 500,000 operating hours, and 69,000 stop-start cycles during a fourteen-year operating history.

One engine had failed four years earlier, and two more engines failed catastrophically over a period of several months, and in spite of investigations by the manufacturers and other parties to the project, no satisfactory root cause was determined.

After the most recent catastrophic event in February of 2011, an investigator was sent to Dhaka, Bangladesh, to investigate the most recent failure.

The debris field was preserved for this investigation and will be shown through diagrams and photographs. The presentation will systematically present the general scene and then guide the discussion to the preliminary findings. A catastrophic failure is characterized by connecting rod separation, ejected pistons, ejected counterweights, fractured blocks, and damage to adjacent engines. A massive failure of this type requires the replacement of the entire block, a very difficult and expensive process, especially in a relatively remote location with logistic challenges. As in many such cases, the preservation of the evidence and failure artifacts is paramount.

A surprising twist occurred in this investigation. Although the investigation was to focus only on the most recent failure, in fact, the two previous failures were re-examined in light of the findings. A common failure mode was then developed, namely hydro-locking of these massive engines on start-up. But what was unknown, and a unique aspect of this work, was that multiple instances of hydro-locking could contribute successively to failures that were occurring seemingly at random.

The presentation will review other contributing factors and phenomena that, although independently would not be seen as causative factors, when confounded proved to be important elements in the failure matrix. The key to linking these events was the chance discovery and examination of artifacts from prior failures.

As in many cases, determined detailed examination of failure artifacts, in this case the cylinder liner, proved that the hydro-locking phenomenon was not a single cycle overload event, but rather that these instances could accumulate from multiple occurrences, and that the time of ultimate failure was not easily predictable. This investigation concluded with evidence of the root cause and methodology that would monitor potential failure modes, as well as preventative procedures to mitigate the likelihood of these multi-million dollar failures.

This presentation will show by photographs the important elements of the fact finding, segregation of the engine debris field, metallurgical failure modes, and the final evidence of multiple hydro-lock events.

Diesel, Hydro-lock, Wartsila

C34 Evaluation of Head Protection Provided by Sports Helmets

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After attending this presentation, attendees will have a greater understanding of sports helmet safety, with the ability to identify helmet designs and materials that can reduce risk of brain injury and concussions in helmeted head impacts.

This presentation will impact the forensic science community by demonstrating the use of a helmet drop test method with a biofidelic head form that can be used to compare helmets to meet different testing standards.

In this study various helmets were subjected to identical drop tests onto a hard flat surface and the resulting head accelerations were analyzed and compared. More than fifteen different helmets of various designs were tested. Helmets used for ice hockey, snowboarding, bicycle riding, skateboarding, football, and motorcycle riding were tested. These helmets are designed to different standards that vary according to sport and country.

Comparison of impact attenuation under the same identical hard surface impact conditions with a confirmed biofidelic head form provided a unique opportunity to evaluate the head protection in potentially serious to fatal head impacts.

Testing Method: The sports helmets were attached to an instrumented head form. The head form utilized was from 50th percentile of a standard instrumented Anthropomorphic Test Dummy (ATD) with a calibrated triaxial accelerometer mounted internally. The helmeted heads were dropped onto a hard flat asphalt surface from a height of 60 inches. The free-fall drops resulted in impact speeds of approximately 12mph. Each helmet was impacted on the top, back, right, and left sides in four separate drop tests. Each test was recorded on high-speed video (1000 frames per second) and real time video. Orthogonal ATD head accelerations versus time were recorded as specified in SAE J211, *Instrumentation for Impact Test*. Resultant head acceleration traces and Head Injury Criteria (HIC) values were compared and analyzed.

Results and Comparison: Due to the common impact velocity, the area under the acceleration curves were comparable and not a significant factor. However, peak head acceleration values and trace shapes exhibited considerable differences. The response differences are analyzed and interpreted.

For comparison, the accelerations and resultants were normalized with respect to helmet weight because the weight of the helmet influences the head acceleration. Helmet characteristics associated with the best impact attenuation were identified.

Since all head injury criteria are related in some way to head acceleration magnitude, helmets that reduce or minimize head acceleration provide better protection. Helmets with thin, soft, or non-existent liners provided the least protection. For example, a novelty type motorcycle helmet exhibited the worst performance, reducing the resultant head acceleration in a top side impact by only about 3% from a comparable bare head impact (487g). These novelty helmets are not safety certified, and although the rider wearing this helmet could circumvent helmet laws in various states, he or she would not be protected from injury in an accident. By comparison, a similarly shaped open face DOT-rated motorcycle helmet reduced the head acceleration by 50%. This reduction was the result of the Styrofoam-type liner's ability to dissipate the energy from the impact.

An even better performing helmet was the ski/snowboarder helmet, which reduced the head acceleration by 64%. Ridges in the shape of the shell deformed and helped absorb energy. However, such ridges would not be acceptable in a motorcycle helmet that needs a low friction, continuously slick hard surface. The best helmet tested was a newly designed hockey helmet that reduced the resultant head acceleration by 66%. This helmet has a liner made of hollow plastic cylinders placed adjacent to one another. The walls of the cylinders deform in the impact. This helmet performed better than an earlier designed hockey helmet with a firm foam liner that reduced head acceleration 60%. Head acceleration pulse shapes are flatter and elongated in all tests where energy is dissipated by the liners.

Conclusions: Based on the testing performed, protection varies widely from one helmet to another. All helmets provide some skull protection. Brain injury prevention requires impact attenuation. The impact attenuation provided is directly related to the liner material properties. Inadequate to non-existent liners, as in novelty helmets, provide little to no brain protection. Some sports helmets with contoured deformable shells can also help dissipate the impact energy. Most impressive is the superior protection provided by the new liner designs that utilize deformable plastic cylinders.

Head Protection, Sports Helmets, Helmet Drop Testing

C35 Analysis of the Parking Garage Vehicular Protection System in a Vehicular Crash Investigation

Daniel M. Honig, PE, Structures Consulting Engineer, PO Box 125, Swarthmore, PA 19081*

The goal of this presentation is to present a case where a vehicle traveling down an exit ramp of a parking garage exited the side of the building and landed top down in an adjacent alley. The investigation into a lack of proper and adequate perimeter safety protection will be described.

This presentation will impact the forensic science community by illustrating the relationship between strength values used in design as compared to the strength at catastrophic failure as well as emphasizing the necessity to properly apply the requirements of adopted building and maintenance codes.

Design load values are based upon the probability that the structural elements will remain in an undeformed condition as a result of an imposed load. Building codes require that the structural attachments of a vehicle barrier system be able to transfer the load to the building's structural elements.

This recent vehicle crash incident occurred on the third level of a parking garage located in a downtown metropolitan area. For unknown reasons, a driver and car traveled down the exit ramp, jumped a concrete curb, and impacted a precast concrete wall panel, thereby

knocking the panel into an alley. As a result of the vehicle loss of control within the garage and the lack of proper and adequate perimeter safety protection, the car exited the side of the building and landed upside down in the adjacent alley.

The original restraint/protective system previously located along the sides of the garage was subsequently removed at the time of an architectural facade renovation in the late 1960s. The existing perimeter system in the garage at the time of the incident was limited to two main components: architectural precast concrete panels attached to the floor slabs and concrete curbing and wheel stops. The combination of the concrete curbing element and the architectural precast wall panel was not able to resist the lateral impact loading of a vehicle traveling at less than fifteen miles per hour, as required by the building code. The architectural facade support system was not a vehicular restraint system. The absence of an additional proper and adequate code-compliant vehicle restraint system allowed the in-place architectural facade support system to fail locally under direct vehicular impact loading.

Multiple field reviews of the existing parking garage building conditions had been performed by various engineering/consulting firms over the course of an approximate thirty-year span beginning in 1978 through the time of the incident. These reviews noted the absence of adequate vehicular protection for the architectural facade system. The building owner, the parking garage management firm, and a construction administration firm were all aware that the exterior precast wall panels were potentially unable to resist vehicular impact loading prior to the incident.

The parking garage management firm and the construction administration firm suggested that the architectural precast concrete panel system was “grandfathered” into the current building code. This “grandfather” description was not correct, given that the current building code specifically stated that existing structures and building elements were not “grandfathered” with regard to general safety and welfare of the occupants and the public. The structural inability of the existing architectural facade panels and/or their connections to adequately support and transfer the code-required vehicular loading posed a threat to the general safety of the parking garage patrons and public use of adjacent surrounding areas at grade.

Structural and civil engineering practice involves the inclusion of normal factors of safety as part of a proper and thorough design process for new and existing building systems and related components. These factors are the relationship between strength values used in a design as compared to the strength at ultimate or catastrophic failure. A properly designed vehicle restraint system would take into consideration load combinations for extraordinary events as well as factors of safety. The calculated catastrophic failure point of the mechanical anchors supporting the architectural panel was significantly less than even the unfactored design values for a vehicle restraint system. Given the use of factors of safety, a vehicle restraint system properly designed would not have had a catastrophic failure until it was subjected to an excessive force.

A proper and adequate code-compliant vehicle barrier restraint system was required to be designed, installed, and maintained in order to properly resist vehicular impact loading by the building code in existence at the time of the original construction of the garage, the building code in existence at the time of the addition of the building atop the garage, and the building code in existence at the time of the preliminary condition appraisal report, as well as the recent and current editions of the property maintenance codes.

Vehicular Impact, Design Load Values, Catastrophic Failure

C36 Forensic Architecture: An Introduction and Case Studies

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After attending this presentation, attendees will learn what forensic architecture is, and how it differs in practice from forensic cases in other fields of science. The presentation will examine how three types of cases were successfully managed.

This presentation will impact the forensic science community by an introduction to forensic architecture with pertinent details of three forensic cases and how they apply to the practice of architecture.

Forensic architecture varies from case to case, but seems to differ from cases in other forensic sciences, as it may involve situations both before and after the occasion of injury, damage, or public disputation.

Situations arising before the occasion tend to be preventive in nature, such as surveys to identify architectural barriers, or the development of standards that serve as guidelines to building safe environments. These cases usually do not become legal situations; however, cases that arise after the occasion of injury or loss due to non-adherence to building codes, regulations and/or construction specifications, usually end up in the legal system.

Case 1: A nationally franchised chain leased a building on the main street in a small Pennsylvania town, made interior renovations, and added a ramp from the public sidewalk to the front door. The ramp was constructed in violation of the building code, and the restaurant owner filed for approval of a variance to allow the ramp to remain. The variance was refused but the owner appealed, received a new hearing, and approval of the variance. A citizen filed a petition to deny variance and the case was reopened. When data was analyzed, the ramp was found to be egregiously in violation of the building code (14.4% slope vs. allowable maximum 8.33%) and not a safe wheelchair ramp. As proof, a center-of-gravity study was presented to demonstrate the danger of allowing the center of gravity of a person using a wheelchair to fall behind the point of support, creating conditions whereby wheelchair users would fall backward. Decision is pending from the Administrative Officer of the Pennsylvania Department of Labor and Industry.

Case 2: A home modification was necessitated by the tragic aftermath of a child’s vaccination, resulting in a little girl suffering from mitochondrial disease, causing developmental delay, seizures, bronchospasm, and an inability to use her limbs. The family, who lives in suburban Atlanta, sued the Secretary of the U.S. Department of Health and Human Services. The Department of Justice undertook resolution of the matter. There was an inspection and analysis of the family’s home. Field-measured drawings, photographs, interviews with the family, review of medical records, and observation of professional therapy sessions provided necessary data. A 3D computer model of the home was created and analyzed to provide a design solution and construction cost estimate of reasonable and necessary home modifications. The modifications included a residential elevator, reconfiguration of several rooms, and use of second-story space above the foyer to accommodate an accessible bedroom and bathroom for the child on the second floor, including a ceiling-mounted, motorized personal transport system to move her from bed to bathroom fixtures. Also provided were a small desk space for a nurse and caregivers, and storage for medical supplies. The garage door opening was raised for access by a wheelchair-accessible van, and ramping added for access to the front door and rear patio. A report was presented to the Special Master who accepted the design and settled the case.

Case 3: In 1993, three weeks before the official opening of the Holocaust Memorial Museum in Washington, a complaint of non-compliance with the Americans with Disabilities Act (ADA) was received from an advocacy organization maintaining that the new museum violated design guidelines of the ADA. The main objection of the organization was the inaccessibility of the sacred central space in the Hall of Remembrance. After study of the construction and upon meeting with the general counsel of the United States Architectural Transportation and Barriers Compliance Board (ATBCB), a design was proposed for a special wheelchair lift carefully integrated into the stairs to preserve the sacred atmosphere of the space. Thus, design issues regarding noncompliance with the ADAwere resolved and the Holocaust Memorial Museum opened according to schedule.

Building, Architect, Construction

C37 Analysis of a Vinyl Railing System in a Second Story Fall Incident

Daniel M. Honig, PE, Structures Consulting Engineer, PO Box 125, Swarthmore, PA 19081*

After attending this presentation, attendees will have been introduced to a case where a fall incident occurred from the second-story exterior rear deck of a vacation residence when a vinyl railing section suddenly and unexpectedly failed at the structural connection to the railing support vertical posts. The investigation into a lack of a proper and adequate railing system will be described.

This presentation will impact the forensic science community by illustrating the need for proper design, installation, inspection, and maintenance of secondary building components, such as railing systems, emphasizing the necessity to properly apply the requirements of adopted building and maintenance codes.

Building codes require that railings/guards have the proper connection devices to transfer load to the appropriate adjacent primary structural framing elements of the building. The railing section involved in this incident had never been inspected throughout its eight-year life span, during which time it was exposed to exterior weather elements.

This fall incident occurred from the second-story rear deck of a vacation residence. The tenant of the vacation residence placed his hand on the vinyl railing of the deck for support and the railing section suddenly and unexpectedly failed at the structural connections to the vertical support posts, thereby causing both the tenant and the railing section to fall to the ground below.

The vinyl railing involved in this incident was installed by the contractor during the original time of construction. An exemplar section of the vinyl railing section was removed from the deck area after the incident. The horizontal railing members of the vinyl rail system consisted of a wood core with a vinyl cover and were toe-nailed with galvanized screws. The manufacturer's instructions called for the installation of coated aluminum brackets fastened to the bottom rail and to the sleeved post.

The manufacturer's documentation specified that the wood cores of the horizontal members be fabricated of cedar wood; however, review of the exemplar railing section confirmed the upper horizontal member consisted of two varying wood sections finger-jointed together and the lower horizontal member consisted of a nonpressure-treated standard framing member. These as-built structural wood cores were not consistent with the specifications, nor were they suitable for the intended use.

The manufacturer's installation guide recommended cutting the vinyl covering of the horizontal handrail members shorter than the wood core to allow for expansion and contraction due to weather. This procedure created an area of vulnerability where the structural wood core was not protected. During inclement weather conditions, moisture would infiltrate the horizontal wood core to vertical post connection locations. This condition continued and was inadequately maintained over time, thereby allowing the significant deterioration to occur.

The jurisdiction where the property is located required a rental license for all rental properties. Although the jurisdiction inspected and reviewed the residence at the time of original construction, according to the jurisdiction's regulations for rental properties, annual inspections of the property are mandated in order to retain a rental license. The property owner never obtained the required rental license.

According to the municipal fire inspector, an annual inspection would have required the property to be compliant with the State Fire Prevention Code. The railing system was required by the building code at the time of original construction of the deck, and the fire prevention code required that all required means of egress safeguards be maintained in good working order. The fire prevention code further required that any exterior means of egress found in a state of deterioration or determined to be unsafe by the fire official be repaired immediately.

The railing system of this elevated exterior deck could not support the code-mandated and expected lateral forces encountered during its lifetime. An experienced contractor should have been aware that toe-nailed connections would not provide adequate structural support without the use of additional brackets or connection mounts. In addition, the galvanized connection screws were subject to deterioration given direct exposure to the weather elements. An experienced contractor should have been aware that no weather-resistant protection between the horizontal structural supports and the vertical wood posts provided a vulnerable place for moisture penetration.

Given the improper as-built construction of the vinyl railing system, the improper connection of the horizontal members to the vertical support posts, and the lack of maintenance and inspection by the owner, the structural connection of the horizontal members to the vertical posts failed under normal and code-required loading, thereby allowing the tenant of the residence to fall to the ground below. The design, configuration, construction, and installation of this railing system, as well as the improper maintenance and lack of inspection by the property owners, allowed this incident to occur. Had this residence been properly licensed as a rental property, the rental unit would have been annually inspected and this incident could have been prevented.

Weather Exposure, Maintenance, Railing Failure

C38 The Effect of Polyethylene Shoe Covers on the Available Friction of Reference Surfaces

Michael A. Sapienza, BS, Fleisher Forensics, 323 Norristown Rd, Ste 130, Ambler, PA 19002*

The goals of this presentation are to demonstrate the effect of an intermediate layer of textured polyethylene material on available friction and also show the value of the protocol described in ASTM F2508 Standard Practice for Validation and Calibration of Walkway Tribometers using reference surfaces.

This presentation will impact the forensic science community by demonstrating the importance of using reference surfaces to evaluate available friction.

Method: ASTM F2508 was used as a reference to measure the effect of polyethylene shoe covers on test results using the prescribed reference surfaces with a validated and calibrated Mark II portable inclinable articulated strut tribometer. The reference surfaces were those included in the ASTM Adjunct to F2508 consisting of ADJF2508-T1 (Vinyl), ADJF2508-T2 (Ceramic), ADJF2508-T3 (Porcelain), and ADJF2508-T4 (Granite). An additional reference surface of 2"x10" pine was incorporated as an exemplar surface encountered on a basement stairway.

Service companies, such as plumbing, heating, H&V, moving, furniture, and flooring, routinely require their employees to wear shoe covers in an attempt to protect their customers' flooring and walkway surfaces from tracked-in soil and moisture. The shoe covers vary in type and some are advertised as waterproof and slip resistant on household walkway surfaces. In particular, certain waterproof, slip-resistant, textured polyethylene shoe covers were used during a routine plumbing repair that resulted in an individual slipping and falling down a flight of 2"x10" pine basement stairs.

To determine the effect of the shoe covers on the available friction of the reference surfaces, adjunct tiles from ASTM were obtained along with the ASTM F2508-11 Standard Practice for Validation and Calibration of Walkway Tribometers using Reference Surfaces. A piece of 10-year-old 2"x10" pine board was also used as an exemplar of the incident stairway. Two materials were evaluated as shoe soling, a standard Neolite® sensor material, and the same material covered with the incident-textured, plastic waterproof shoe cover.

Forty tests for each surface/sensor condition were conducted: 10 in each of 4 orthogonal directions, that is, at 0°, 90°, 180°, and 270° relative to an arbitrarily defined direction on the reference surface. A total of 400 tests were conducted (10 replications x 4 directions x five surfaces [sample] x two sensors [columns]). The tests were analyzed using ANOVA: Two Factor with Replication. Direction was not included as it had been previously found to be insignificant. Surface [Sample] and Sensor [Columns] were both highly significant (*p*-values of 1.9E-142 and 0.00 respectively).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample [Surface]	2.86855	4	0.717138	435.8955	1.9E-142	2.394824
Columns [Sensor]	26.91534	1	26.91534	16359.87	0	3.865413
Interaction	0.080276	4	0.020069	12.19848	2.36E-09	2.394824
Within	0.64163	390	0.001645			
Total	30.5058	399				

For the 40 tests on each reference surface, the mean, Standard Deviation (SD), Standard Error (SE) of the mean, and 95th percentile Confidence Interval (CI) for the walkway tribometer test results were calculated. Using the mean and standard deviation, paired *t*-tests determined statistically significantly different results (t >1.694) for both sensors on all reference surfaces. (Neolite® — shoe-covered Neolite®).

Granite using standard Neolite®			Granite using shoe covered Neolite®			Paired t-test difference	
Mean	1.00		Mean	0.47		Mean Diff	0.529
Std Dev	0.000		Std Dev	0.045		Std Dev	0.045
Std Error	0.000		Std Error	0.007		T	74.403
95% CI	1.000	1.000	95% CI	0.48542	0.45758	t-test	>1.694
Porcelain using standard Neolite®			Porcelain using shoe covered Neolite®			Paired t-test difference	
Mean	0.833		Mean	0.333		Mean Diff	0.500
Std Dev	0.053		Std Dev	0.038		Std Dev	0.048
Std Error	0.0084		Std Error	0.0060		T	65.607
95% CI	0.84976	0.81674	95% CI	0.34477	0.32123	t-test	>1.694
VC tile using standard Neolite®			VC tile using shoe covered Neolite®			Paired t-test difference	
Mean	0.798		Mean	0.283		Mean Diff	0.515
Std Dev	0.091		Std Dev	0.012		Std Dev	0.094
Std Error	0.0143		Std Error	0.0020		T	34.830
95% CI	0.82612	0.76988	95% CI	0.28659	0.27891	t-test	>1.694
Ceramic using standard Neolite®			Ceramic using shoe covered Neolite®			Paired t-test difference	
Mean	0.996		Mean	0.513		Mean Diff	0.483
Std Dev	0.014		Std Dev	0.015		Std Dev	0.018
Std Error	0.0021		Std Error	0.0023		T	173.678
95% CI	1.00044	0.99206	95% CI	0.51756	0.50844	t-test	>1.694
2 x 10 Pine using standard Neolite®			2 x 10 Pine using shoe covered Neolite®			Paired t-test difference	
Mean	0.900		Mean	0.333		Mean Diff	0.567
Std Dev	0.033		Std Dev	0.016		Std Dev	0.041
Std Error	0.0052		Std Error	0.0026		T	86.950
95% CI	0.90979	0.88921	95% CI	0.33781	0.32769	t-test	>1.694

It was clear the presence of the textured polyethylene plastic, waterproof shoe covers lowered the available friction of the reference surfaces and presented a slip hazard to the individual wearing them.

An additional paired t-test was done using the inside surface of the shoe cover as the walkway surface and standard Neolite® as the sensor under wet and dry conditions to determine the effect of moisture on the inside of the shoe cover.

Shoecover using std Neolite® dry			Shoecover using std Neolite® wet			Paired t-test difference	
Mean	0.616		Mean	0.089		Mean Diff	0.527
Std Dev	0.025		Std Dev	0.018		Std Dev	0.027
Std Error	0.0039		Std Error	0.0028		T	123.577
95% CI	0.62343	0.60807	95% CI	0.09401	0.08299	t-test	>1.694

The shoe cover also presented a hazard to the wearer if used over wetted shoe soles.

Shoe Covers, Slip Resistance, Reference Surfaces

C39 Analysis of ASTM F2508 Reference Surfaces Using Logistic-Regression-Based Criteria

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The goal of this presentation is to inform the attendees how to qualify reference surfaces and the implications of the same. ASTM 2508 is a new standard designed to qualify Walkway-Safety Tribometers. This paper uses logistic regression to analyze the quality of the reference surfaces.

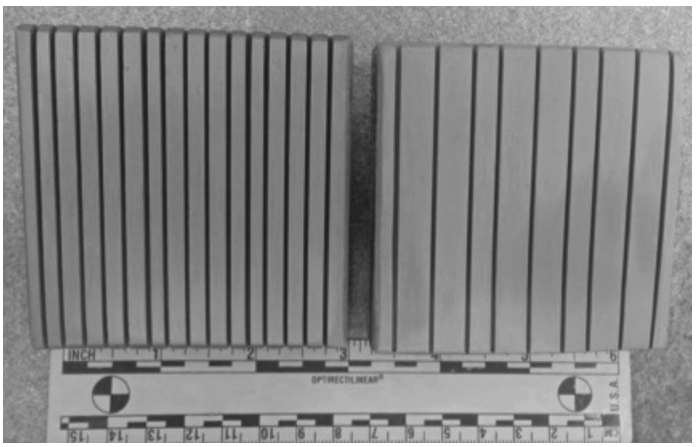
This presentation will impact the forensic science community by showing how the nuanced view of the ASTM F2508 reference-surface qualification methodology for tribometers will allow researchers and practitioners to have more than a one-dimensional "handbook" view of tribometric-test variability and reliability. This is essential for an understanding of the relationship between fall hazard and tribometric test results.

Background: The measurement of the available friction between the floor surface and a shoe (or foot) bottom is of significant import in determining pedestrian-ambulation safety. High-school and elementary college physics, for the sake of simplicity, assume the Amontons-Coulomb friction model, where it is axiomatic that friction is solely a function of the materials in contact, and independent of contact pressure, velocity, temperature, and so forth. This model holds reasonably well when the materials are non-resilient, e.g., steel and/or wood. The model is often problematic when one of the surfaces is resilient, like a shoe or foot bottom, and essentially irrelevant when the interface between the materials is lubricated. In that situation, factors other than the materials involved become significant. Importantly, the friction-measuring instrument itself (called a Walkway-Safety Tribometer, or WST) becomes a factor in the measurement. That is, different WSTs, measuring the same surfaces, will give different, sometimes very different, results. This is caused by two factors: (1) on a fundamental level, different WSTs use different friction-generating mechanisms (for example, a drag-sled or articulated-strut tribometer cannot replicate the hydrodynamic, squeeze-film phenomenon that occurs when pedestrians slip on a wet surface); and, (2) assuming that the WST is capable of replicating the appropriate friction mechanism, the parameters (especially pressure/time effects) are different, both between different models of WSTs, and different between a WST and an ambulating pedestrian. Because the ultimate purpose of WST test results is to determine if a walking surface (or shoe bottom) conveys adequate safety, the test of utility for any WST results are how they reflect pedestrian-ambulation traction. The concept that WST tests must ultimately reflect on pedestrian-ambulation safety is called biofidelity. Recognizing this, and troubled by the fact that the then-current ASTM WST standards referenced proprietary instruments, the Board of Directors of the ASTM convened a task group under the guidance of a former ASTM Chairman of the Board to address this issue. From this, ASTM-sponsored research at the University of Southern California examined the relationship between a pedestrian's utilized friction and the results of tribometric tests. The end result was a set of four reference surfaces, which are used to "standardize" the tribometer readings. These four test surfaces consist of (from the most to least slippery): (1) polished granite lubricated by a diluted non-ionic solution); (2) Porcelanosa Ferrokor tile, lubricated by distilled water; (3) Official Vinyl Composition Tile (OVCT), lubricated by distilled water; and, (4) unglazed ceramic tile, lubricated by distilled water. The calibration of tribometers using these tribometric reference surfaces is governed by ASTM standard F2508, which was first approved in 2011.

Because walkway-safety tribometry test results are standardized by these four tribometric reference surfaces, it is useful to be able to answer questions concerning the reference surfaces and their relationship to the tribometers. F2508 requires a WST to discriminate—with statistical significance—between the four reference surfaces. F2508

discrimination is verified with t-tests of the adjacent surfaces: granite vs. Porcelanosa; Porcelanosa vs. OVCT; and OVCT vs. unglazed ceramic. There was interest to see if the quality of the reference surfaces using the Slip-Test Mark II portable inclinable articulated-strut tribometer (the PIAST) and logistic regression, which was chosen as an analysis tool because the PIAST gives dichotomous (slip or no-slip) results could be better assessed.

Experiment: A PIAST was modified by fabricating and installing a mechanism to be able to tilt the mast with an order of magnitude of more accuracy than an unmodified PIAST and installed a platform for a high-precision level (0.1 degree repeatability). For the experiments, the PIAST was shod with two slightly different test feet, both of test liner, but with different siping patterns:[1]

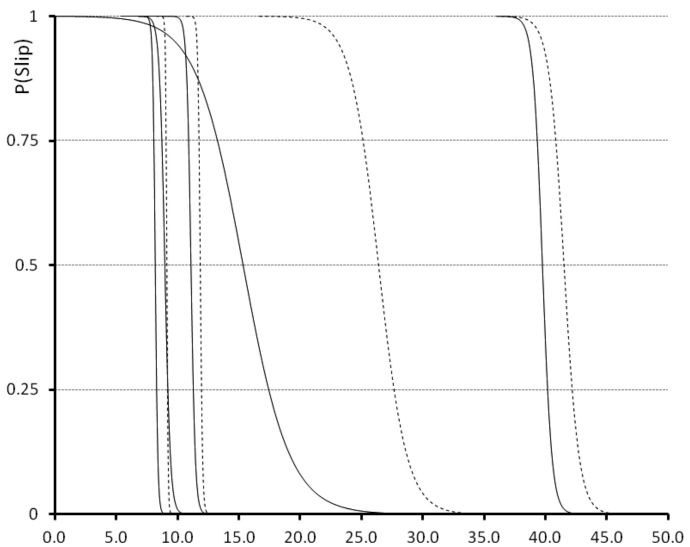


For each reference surface, the PIAST was set to a friction angle low enough so that the test foot would not slip, and the friction angle was slowly increased, typically at a fraction of a degree at a time, taking numerous (typically 25) tests at each friction angle, until slips were observed at 100% of the readings.[2] This was done with each of the two test feet. For each test-foot/reference-surface combination, between 100 and 400 friction tests were taken.

Logistic regression was used to determine the probability of a slip at a given WST setting. Ideally, the WST will always slip at exactly the same value, yielding a step function graph:

$$P(\text{Slip}) = \begin{cases} 1; & \mu \leq \mu_c \\ 0; & \mu \geq \mu_c \end{cases}$$

In reality, because slipping is—in walkway-safety tribometry as in real life—a probabilistic event, the curve obtained is s-shaped: a logistic curve. The steeper the curve, the more like a step function, and the more discriminating the reference surface. The P(slip) = 50% point on the curve is the nominal friction value of the test-foot/reference-surface combination. Here are the results:



Discussion: Visually, it is clear that the granite/diluted non-ionic solution (the leftmost three curves) and Porcelanosa/distilled-water (the fourth and fifth from the left) reference surfaces demonstrate excellent discrimination; the behavior is very close to the ideal step function for the slip/no slip condition. The test-foot-to-test-foot differences for these surfaces were also minimal. The ceramic tile (the rightmost two curves) are not quite as discriminating, but as the friction angle for the slip/no slip dichotomy is quite large (above 40°), the relative variation (analogous to the coefficient of variation for a continuous variable) is small. Problematic is the OVCT, the third and fourth curves from the right. They are both quite shallow, indicating poor discrimination; importantly, the two curves for the two test feet are widely separated, indicating that different (but similar) test feet will give disparate results.

Neither of these OVCT flaws necessarily negates the utility of F2508. As far as the lack of discrimination inherent in the OVCT, one can increase the sample size (an operator checking his equipment will typically perform 12 repetitions on each surface; a validation run requires 40 repetitions on each surface) to compensate for the increased relative variation. As far as the separation between the curves, one of the main purposes of F2508 standardization is to allow results from different tribometric instruments to be compared; this can obviously extend to different test feet on the same tribometric instrument, different operators, and so forth.

That said, it is recommended that the ASTM subcommittee that maintains F2508 (ASTM Committee F-13 on Footwear and Pedestrian/Walkway safety's subcommittee 13.1 on traction) seek to revise F2508 by researching and qualifying at least two other reference surfaces to use in the stead of OVCT.[3]

References:

1. In the graph (above), the test foot on the left is represented by the dashed curves. The test foot on the right is represented by the solid curves.
2. The friction angle is the angle of inclination of the PIAST mast. The friction coefficient is the tangent of the friction angle.
3. It is recommended that ceramic reference surfaces both having low variability and in the range of 20° and 30° friction angles be explored.

Forensic Science, Tribometry, F2508

D1 DNA Identification of Mass Disasters' Victims and Ethical Issues

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After attending this presentation, attendees will better understand the ethical aspects concerning the activity of identification of victims of mass disasters using DNA technology.

This presentation will impact the forensic science community by not only stressing the importance of DNA identification of victims of mass disasters, but also using caution with ethical issues with the potentially sensitive nature of DNA identification.

A mass disaster is an unexpected event that causes serious injury and death to a number of people. It may be a natural disaster (e.g., earthquake, volcano, avalanche, hurricane, and tsunami), an accidental disaster (transportation accidents and building fires), or an intentional terrorism act (e.g., terrorist activity, war, or political crisis).

Victim identification is difficult but necessary. Proper identification is necessary to notify the legal next of kin, resolve estate issues and criminal/civil litigation, and issue death certificates.

DNA profiling is increasingly becoming an important tool in the individual identification in the aftermath of mass disaster. While forensic geneticists are often not included as first responders, DNA sample collection and a strategy for DNA-based victim identification needs to be part of the community's preparedness plan. In fact, the preparedness plan of the laboratory needs to include policies for family notification, long-term sample disposition, and data archiving.

Many of the decisions which involve prioritization are made daily and often without much thought to the ethical and allocation considerations underlying them. Experienced investigators making these types of decisions are usually deciding about priority for, rather than access to, services or interventions.

One of the objectives of the forensic investigation of human remains is to identify the remains and, if possible, return them to the family of the dead person. This objective helps family members by ascertaining the fate of their relative and allowing the remains to be handled in a culturally appropriate manner, thus enabling the families of the missing to mourn their loss.

Ethical issues are associated with the use of DNA identification, because information contained in a person's DNA is sensitive and it is a unique identifier and may contain information about a person's family and intimate associations. It is widely held that the death of a person does not extinguish the interests of that individual. Indeed, family members, and others who have physical possession or access to an individual's body, tissue, or cells, have to respect certain obligations and rights following the death of the individual.

Although the ultimate goal is to obtain a match between two persons or between a biological material and a person, the specific context of each of these applications of human identity testing has its specific problems, ranging from technical approach, through statistical interpretation, to ethical issues.

There has been very little systematic effort to identify and analyze the major ethical and policy challenges associated with this new use of genetic technology. This study seeks to define some of these ethical aspects focusing on particular situations.

International law does not have any specific provisions for protecting genetic data in mass disaster. International humanitarian law and international human rights law recognize the need to provide special protection for persons affected by armed conflict. However, these bodies of law contain only general principles relating to confidentiality, privacy, non-discrimination, and human dignity that can be applied to the protection of genetic data.

It seems important to evaluate how to deal with resource allocations, incidental findings, ethical acceptance of secondary uses of the biological materials collected, and the use/misuse associated with the creation of large genetic databases. Last but not least, the issue of privacy, which has to be rigorously protected to ensure the respect of human individual rights must be addressed.

DNA Identification, Mass Disaster, Ethics

D2 Advantages of Forensic Anthropologists' Involvement in Mass Disaster Responses: A Review of the I-75 Mass Fatality

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After attending this presentation, attendees will learn how anthropological methods can aid in the recovery and identification of individuals in a mass disaster event involving multiple vehicle collisions. A systematic interagency approach using archaeological methods maximized the recovery of skeletal elements and personal effects necessary for identification with minimal damage resulting from the recovery process.

This presentation will impact the forensic community by demonstrating the utility of forensic anthropologists in the recovery of remains from burned and altered vehicles. The archaeological approaches used by forensic anthropologists are extremely useful in mass disasters, particularly when remains are burned, fragmentary, and commingled.

On January 29, 2012, a multi-vehicle crash occurred on Interstate 75 in the Gainesville, Florida, area as the result of reduced visibility due to fog and smoke from nearby wildfires. The crash resulted in 11 deaths and at least 23 injured. The District 8 Medical Examiner's Office consulted forensic anthropologists from the C.A. Pound Human Identification Laboratory at the University of Florida to assist in the recovery of victims within the burned vehicles. Two of the vehicles involved were towed to a secure location at the Alachua County Sheriff's Office for processing. The first vehicle contained the remains of a single individual. Although the majority of this individual was removed by medical examiner personnel with the assistance of an anthropologist at the scene of the accident, the forensic anthropologists present were able to quickly sort through the debris in the car to recover any remaining burned bone fragments.

After completion of the first vehicle, the search and recovery inside the second vehicle, a small pickup truck, began on the afternoon of January 30, 2012, and continued through the morning of February 2, 2012. The truck was severely damaged with the roof, dashboard, and

back wall of the cabin collapsed inward toward the seats. Due to the condition of the second vehicle from both the accident and subsequent vehicle fire, vehicle identification information was not accessible until the contents of the vehicle were removed. Although burned remains were evident in the passenger and driver seats, the total number of individuals inside the truck was unknown at the start of recovery. As the recovery progressed, local fire and rescue personnel assisted in removing portions of the truck cab to facilitate access inside of the vehicle. The processing of this vehicle required the modification of traditional archaeological methods. Separate areas of the vehicle (driver seat, passenger seat, backseat, console area) were defined to minimize further commingling of fragmentary remains during the recovery process. All material was removed by hand and screened using 1/8" or ¼" screens. As trained osteologists, the forensic anthropologists on scene were quickly able to distinguish between burned remains and other visually similar materials, including wood, insulation, car seat foam, and other melted and altered aspects of the car interior. In general, the skeletal remains were burned and highly fragmentary, exhibiting black to white coloration. Anthropologists and odontologists examined dental fragments recovered for developmental age and restorations that could facilitate a more rapid identification, with fixation used as needed. Other artifactual evidence useful for identification, including, jewelry and paper materials with writing, were recovered with minimal additional damage to these items. All osseous material upon removal was photographed, bagged, and labeled with the provenience (i.e., associated area of recovery). Debris determined to be non-osseous or non-evidentiary was placed in a designated area separate from bone and other material collected. Additionally, the recovery process was fully documented through photography of the vehicle during excavation.

Ultimately, three individuals were recovered from the vehicle: one in the driver's seat, one in the passenger's seat, and one in the backseat. Maintaining provenience at all times, including separate excavation areas within the vehicle, documentation, and transport to the medical examiner's office, allowed for minimal commingling of remains during recovery. Systematic screening and clearing of all debris in the vehicle resulted in the recovery of as many skeletal elements as possible, as well as personal items, which aided in the rapid identification of those individuals in the vehicle. This incident highlights the importance of interagency collaboration and the inclusion of appropriate specialists to efficiently and effectively recover and identify individuals in a mass disaster event.

Anthropology, Mass Disasters, Burned Remains

D3 Isotope Forensic Evaluation of Modern Human Remains From the University of Tennessee William M. Bass Donated Skeletal Collection

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After attending this presentation, attendees will gain a greater appreciation of isotopic patterns in the modern American population derived from enamel, hair, and bone, and also an introduction to novel sample preparation techniques.

The presentation will impact the forensic science community by presenting multiple isotopic values of bone collagen, bioapatite (phosphate and carbonate), and hair keratin from the William M. Bass Donated Skeletal Collection (WBDSC), the largest modern skeletal collection in the United States.

The present study builds on recent advances in isotopic forensic research by examining multiple isotopic variations in the William M. Bass Donated Skeletal Collection (WBDSC). The WBDSC represents the largest modern skeletal collection in the United States and the collection is housed at the University of Tennessee Forensic Anthropology Center (UTK FAC). For this study, the FAC Donations serve as a proxy for modern surface and buried forensic cases.

Multiple constituents including bone collagen, bioapatite (phosphate and carbonate), and hair keratin from 102 donations were prepared for $\delta^{13}C$, $\delta^{18}O$ and $\delta^{2}H$ analysis using refined protocols. The protocols were enhanced by shortening the cycle of each sample preparation period for collagen extraction utilizing a filter-bag method assisted by an ultrasonic water bath, and modifying Thermo TC/EA for improving analysis precision of phosphate $\delta^{18}O$.

Dental enamels were sampled using a NewWave Micromill and analyzed for $\delta^{18}O$ of phosphate. The averaged $\delta^{18}O$ value was 17.50±1.36‰ VSMOW (n=45). Correlation analysis of the dental $\delta^{18}O$ values with meteoric water $\delta^{18}O$ at birth location (modeled) yielded the equation ($\delta^{18}O_{tooth}=0.62\delta^{18}O_{water}+21.74$, r=0.63, n=45), which was very similar to the equation ($\delta^{18}O_{bone}=0.64\delta^{18}O_{water}+22.37$, r=0.98) generated by Longineli (1983).

Non-exchangeable $\delta^{2}H$ of hair keratins were also analyzed using Thermo TC/EA. The averaged $\delta^{2}H$ was -83.35± 6.36‰ VSMOW (n=14). The $\delta^{2}H$ values exhibit a positive correlation with the meteoric water $\delta^{18}O$ at death location (r=0.68), and a negative correlation with altitude (r=-0.73) that is consistent with isotope "Altitude Effect." In addition, 61 bioapatite carbonates were analyzed for $\delta^{13}C$ and $\delta^{18}O$. The averaged value was -9.32±1.31‰ (VPDB) for $\delta^{13}C$, and -6.91± 1.41‰ (VPDB). But there was no meaningful geographical information inferred from these bone apatite samples. Bone collagens (n=77) were extracted for N, C, N/C ratio, $\delta^{13}C$ and $\delta^{15}N$ analysis. The averaged carbon content in human phalanx bone collagen was 34.55±5.20%, 12.60±2.05% for nitrogen content. The averaged C/N ratio was 2.76±0.21. The averaged $\delta^{13}C$ value was -15.72±0.9‰ (VPDB), and 11.12±0.51‰ (AIR) for $\delta^{15}N$.

This study indicates that the dental enamel $\delta^{18}O$ values from the WBSC collections are overall reflective of the individual's birth location, whereas hair keratin $\delta^{2}H$ values are influenced by the individual's death location, which is consistent with several other isotopic studies of forensically derived human samples and suggests that the application of the dual isotope (O, H) could provide better constrain on the residential history by pin-pointing the beginning (tooth) and the ending (hair) of the individual life journey. Although the correlation coefficient of the dental $\delta^{18}O$ with local water is not as high as reported by several other researchers, the relationship; however, does follow the trend of the earlier study.[1] The potential influence of isotopic pattern of tap water will also be discussed to examine the variability seen in the WBDSC sample.[2] It is suspected that the WBDSC does not represent a more geographically heterogeneous sample and it is likely that self- or family-reported residential histories as is the practice at the UTK FAC are more variable.

This project is supported by the National Institute of Justice, Office of Justice Programs, Grant #2008-DN-BX-K193.

References:

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Stable Isotope, Tennessee, Human remains

D4 Fatal Dog Bites in Children: Three Cases From Central Virginia

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After attending this presentation, attendees will have a better understanding of the circumstances surrounding child fatalities due to dog bites in central Virginia.

This presentation will impact the forensic science community by providing a generalized description of child fatalities due to dog bites in central Virginia, which may prove useful in determining how to prevent fatal dog bites in children and determining when situations involving dogs and children can prove more likely to have a fatal outcome.

In the past twelve years, three cases of fatal dog bites involving children have been investigated. The similarities and differences between these cases will be discussed in three separate case reports.

The first case occurred in 2000 involving a 6-year-old white male who was airlifted to a hospital and later died after suffering lethal dog bites to the head and neck. The victim had gone outside by himself to feed the family dog. The bites to the head were so severe that part of the skull became visible. The dog involved in this death was a wolf-German shepherd mix.

The next two cases were more recent. The case from 2009 involved a 1-year-old white female who was left alone and wandered outside to where her family's dog was chained. She sustained multiple puncture wounds to the neck. The dog involved in this attack was a pit bull.

The third case from 2011 involved a 5-month-old white male who had been left alone in the same room as the family's dog. The victim was found unresponsive after family heard growling. The victim sustained multiple lacerations to the forehead. The dog involved in this death is a rescued bulldog.

In all three cases, the events were not witnessed. Additionally, all the victims were last seen alive shortly before the attack and were not found until the child either cried out during the attack or family members heard or noticed something out of place and found the child right after the attack. In all three cases, the trigger for the attack was unknown. Three similarities emerged between the three cases: (1) all three dogs are stereotypically labeled "attack" breeds; (2) the dogs involved had no known previous violent history and no previous incidents of aggression; and, (3) all three victims had injuries to the head and neck areas. One difference between all three cases was the age difference; the victims ranged in age from five months to six years.

Further investigation into these cases will examine the living situations of the families involved, the type of dog involved in the attack, the location of the incident, and the location, depth, and severity of the injuries. Future investigation will include investigation of fatal dog attacks from the other three districts of the Virginia Medical Examiner system. The presentation of these three cases from the central region of Virginia will eventually be part of a larger study of fatal dog attacks involving children in the entire state of Virginia.

Dog Attack, Fatal, Child Investigation

D5 Medicolegal Aspects of Forensic Investigations of Mass Graves in Katyn (1940) and Tuskunenu (1947)

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After attending this presentation, attendees will learn about medicolegal means used in the investigations of the Katyn and Tuskulenu massacres. This presentation will compare medicolegal methods used in these investigations.

This presentation will impact the forensic science community by addressing medicolegal means used in the investigations methods used in Katyn and Tuskulenu massacre investigations.

The Katyn massacre, known as the Katyn Forest massacre, refers to mass execution of Polish officers carried out by the Soviet secret police NKVD (The People Commissariat for Internal Affairs) in April – May 1940. The decision was made by the Politburo and signed by Joseph Stalin. The number of victims was estimated at about 22,000, the most commonly cited number being 21,768.[1-4]

The executions in Vilnius discovered at Tuskulėnų were carried out by a special group of NKGB/MGB staff (The People's Commissariat for State Security/The Ministry of State Security). According to gender, 762 men and five women were executed and their ethnic origins were documented. In this study, two medicolegal reports were analyzed.[5,6]

The goal of the study was to analyze by medicolegal means the Katyn and Tuskulėnų massacres and to compare two medicolegal reports of the Katyn massacre, the Germans and Burdenkos Special Commission reports in Katyn. In addition, the German reports consisted of three independent examinations. In Tuskulėnų, all of these medicolegal findings and reports are convergent and the exhumation at Tuskulėnų was conducted in 1994.

The study includes historical documents and references to the Katyn massacre.[1-4] Based on the literature, the author compares both of Katyn's medicolegal reports. The Tuskulėnų massacre was analyzed by medicolegal aspects based also on literature which includes historical documents and references, and the author measurements of the skull injuries.[5] The study analyzed medicolegal methods which had been used in Katyn and the modern medicolegal method which had been used in Tuskulėnų massacre investigation.

The Katyn massacre had been well organized and had taken a longer time than the Tuskulėnų massacre, which had been done intensively and very fast. Killing processes had been done almost similarly on both mass graves. In Katyn, there had been bullets used of calibers less than 8mm, i.e., 7.65mm or less; in a few cases, there was a caliber of more than 8mm, i.e., 9mm in the Katyn massacre.[1] The caliber of bullets were from 6.0 to 9.0 cases, with quadrangular 3 × 3cm entrance and 0.5 × 0.5cm in Tuskulėnų.

Medicolegal examination had been used in both massacres, including modern computerized methods in Tuskulėnųs report and manual methods in Katyn. Medicolegal investigation of Katyns was conducted during the Second World War.[1,2,5,6] In Tuskulėnų, investigations were carried out during the independence time of the Lithuanians without any political pressure.

In conclusion, both the Germans and Burdenkos reports were well organized and professionally conducted; however, they reflect strict political views including propaganda speculation.

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1. BBC International Reports (Former Soviet Union): Russia and the Katyn Forest Massacre 13, 2005.
2. Benedict Humphrey Sumner (1893-1951) Historian, scholar of modern diplomacy and international relations, took part in the Paris Peace Conference as Assistant Secretary. Appointed to the International Labor Office, toured Poland and the Baltic States in 1921. Drawn to Russian history and literature, became an authority on the subject. Fellow and Tutor in Modern History, Balliol College,

Oxford 1925-44, engaged in the Foreign Research and Press Department (Russian Section) of the Royal Institute of International affairs of All Souls College, Oxford 1945-51. Published: Russia and the Balkans 1870-1880 in 1937; War and History in 1945; Peter the Great and the Ottoman Empire in 1949.
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Katyn, Tuskulenu, Massacre

D6 Perurac Lake, Bosnia: A Multidisciplinary Operation to Locate, Recover and Examine DNA Samples, and Identify the Missing From Balkans Conflicts

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After attending this presentation, attendees will become aware of logistical and operational issues in locating and recovering human remains from a riverine environment, the complications these conditions create for examinations, and the impact such an environment has upon relative rates of success of DNA amplification from bone.

This presentation will impact the forensic community by illustrating the successful application of a scientific investigation process from search to identification. The efficacy of DNA analysis from human remains that have been taphonomically altered by fluvial activity over a 20 year period is demonstrated. Moreover, the anthropological re-association of remains, led by DNA linkages, combined with contextual site assessment, missing person's lists, and witness accounts provide identification and can potentially support or refute past behaviors and investigative interpretation.

Perucac Lake has been a presumed site of deposition for numerous individuals, as a result of conflicts in the region. The Lake was formed by damming the Drina for hydro-electricity. In the summer of 2010 when water levels were lowered, a malfunction in the turbines of the dam forced authorities to dramatically reduce the level of the water to enact repairs. An opportunity arose to survey a significant section of the exposed land surface with human remains observed on the banks and riverbed. A multi-national, multi-agency effort was launched with the goal of locating, identifying, and recovering as much as possible.

With the International Commission on Missing Persons (ICMP) assistance, line searches and manual excavations were conducted along on both sides of a 50 kilometer length of the lake and river from Visegrad Bridge to Bajina Basta dam. Approximately 2.5 million square meters were searched over a period of two-and-a-half months in topography along the river that varied from steep canyons to flat beach-like coastlines and mudflats.

Approximately 450 cases varying from a single bone fragment to large quantities of mixed bones, were located, recorded, and recovered from land within four municipalities in two countries: Visegrad, Srebrenica, and Rogatica in Bosnia and Herzegovina; and, Bajna Basta in Serbia. Following preliminary anthropological examination, the Minimum Number of Individuals (MNI) for all recovered cases was established to be 97, based on the right femur.

Remains recovered in Bosnia and Herzegovina were sent to Visoko Mortuary, where ICMP participated in bone sampling for DNA analysis. The remains recovered in Serbia were sent to the Forensic Medicine Institute in Belgrade, Serbia, where they also underwent bone sampling for DNA analysis. The variation in success in extracting DNA samples from different skeletal elements was statistically examined to provide guidance for future sampling strategies for similar investigations.

As of July 2012, 220 unique DNA profiles had been obtained from all the recovered cases, with anthropological re-assessments, guided by DNA profiles, re-associating body parts or single bones to identify individuals. Contextual and evidence analysis also allowed cases to be differentiated between recent conflict from those of the conflicts of previous eras. The investigation and results of the scientific process demonstrate the potential to recover remains and identify the missing from a difficult search environment after many years.

Multidisciplinary, Riverine Environment, Identification

D7 Assisted Suicide and the Next of Kin Rights

Bethany L. Bless, MS, Harris Co Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will learn the definition of assisted suicide and become familiar with the current laws governing it. Legal rights of the next of kin in relation to disposition of remains in Harris County will also be discussed.

This presentation will impact the forensic science community by increasing understanding of laws pertaining to assisted suicide and potential problems in determining the legal next of kin for disposition of remains.

Assisted suicide, also known as euthanasia, is defined as the aiding of another person in his/her suicide. The topic of assisted suicide was brought into the public eye in the 1990s with the criminal court cases against Dr. Jack Kevorkian who was charged multiple times with assisting terminal patient's death via physician-assisted suicide. After several unsuccessful attempts to convict Dr. Kevorkian, the Michigan legislature enacted a law in 1998 making assisted suicide a felony punishable by a maximum five-year prison sentence or a $10,000 fine. On March 26, 1999, a jury in Oakland County, Michigan, convicted Dr. Jack Kevorkian of second-degree murder and the illegal delivery of a controlled substance. That April, he was sentenced to 25 years in prison with the possibility of parole. Many states have also adopted laws against assisting in a suicide, Texas being one of them. Texas Penal Code 22.08 outlines assisted suicide as a criminal offense, although charges rarely are filed. Under this code, a person commits an offense if, with intent to promote or assist the commission of suicide by another, he aids or attempts to aid the other to commit or attempt to commit suicide, a misdemeanor offense. Should serious bodily injury occur to the person being assisted, the offense becomes a state felony.

The first assisted suicide case in which an individual was charged with a criminal offense in Harris County occurred in 2012. Afterward, questions arose concerning the rights of an individual to act as legal next of kin for disposition of remains if they have been charged with assisted suicide of the decedent. In Harris County, the right of a next of kin for disposition of remains is determined by Texas statutes which outline the legal succession of next of kin. According to the Texas Health and Safety Code 711.002, a person may not control the disposition of the decedent's remains if, in connection with the decedent's death, an indictment has been filed charging the person with a crime under Chapter 19, Penal Code, that involves family violence against the decedent. Assisted suicide is a charge under Penal Code Chapter 22 Assaultive Offenses, not under Chapter 19 Criminal Homicide, so there is currently no restriction of the next of kin rights to disposition of the decedent's remains, despite the pending felony charges.

Most American states currently have statutes explicitly criminalizing assisted suicide. Laws need to accommodate the matter of the next of kin's right to dispose of remains if they have been criminally charged in connection with that individual's suicide.

Assisted Suicide, Criminal, Next of Kin

D8 Histomorphology and Age Estimation of the Human Rib Cortex in Methamphetamine Users

Margaret Streeter, PhD, Dept of Anthropology, 1910 University Dr., Boise, Idaho 83725; and Robert C. Karinen, MA, Ada County Coroner, 5550 Morris Hill, Boise, ID 83703*

After attending this presentation attendees will have an understanding of observed histomorphological changes in the bone microstructure of methamphetamine users. In addition, this presentation will demonstrate how methamphetamine use may result in less accurate histological age estimations

This presentation will impact the forensic science community by demonstrating that methamphetamine abuse can be associated with changes to the bone microstructure. This has important implications for those doing histological age estimations using osteon population densities.

Histological age estimation using bone microstructure is an accepted and accurate tool when trying to determine age at death in unknown skeletonized human remains. Studies have demonstrated lifestyle choices such as poor diet, insufficient exercise, and substance abuse can negatively affect bone health. Of these lifestyle choices, the advanced decay of the dentition and pathologies associated with the oral cavity seen in chronic methamphetamine users is often noted. The purpose of this analysis is to determine if the tissue pathology associated with long-term methamphetamine use is a localized response to poor dental hygiene or an indication of a more systemic response that is discernible in the bone microstructure. Studies have associated chronic methamphetamine abuse with lower bone densities in both the axial and appendicular skeleton but to date no studies examine what methamphetamine's role may be on impacting the microscopic structure of bone.

A comparison of the fourth rib cortical bone microstructure between males that were known to be methamphetamine abusers (N=18) and individuals who did not abuse the drug was undertaken (N=19). Histomorphometric variables calculated in this analysis included mean osteon size, Osteon Population Density (OPD), and cortical area measurement. OPD and mean osteon size were found to vary significantly between study and control populations. Users of the drug had a mean OPD of 15.19. Non-users were shown to have an OPD of 21.68. Osteon population densities were found to be significantly less in methamphetamine users ($p<.01$). Users had an average mean osteon size of .041mm^2 compared to non-users at .039mm^2. Mean osteon size was found to be significantly larger in methamphetamine users at a 90% confidence interval ($p<.10$).

When histological age estimations were attempted using Stout and Paine 1992 formula, it was found that individuals who abused methamphetamine, as well as the control group, tended to have age estimations that underestimated actual age. When calculated, the methamphetamine group underaged individuals by an average of 11.6 years. This differed from the control population which on average underestimated ages by 7.1 years. This study has important implications when histological age estimations developed from OPD are conducted on methamphetamine users. As demonstrated, estimated ages for those that abuse the drug are less accurate then non-users. This study demonstrates methamphetamine abuse does affect bone microstructure and impacts the forensic science community by demonstrating that histological age estimations on unknown skeletonized human remains may be less accurate if the decedent abused methamphetamine.

Age Estimation, Skeletal Analysis, Methamphetamine

D9 Evaluation of Clandestine Methamphetamine Diffusion Through Building Materials by Ion Mobility Spectrometry and SPME-GC/MS

Holly A. McCall, BS, 433 Wilson Ave, Apt 4, Morgantown, WV 26501*

After attending this presentation, attendees will have a better understanding of clandestine methamphetamine laboratories and the contaminates that transport through structures where these exist.

This presentation will impact the forensic science community by demonstrating how current standards for clandestine methamphetamine laboratory remediation are not sufficient.

Portable Ion Mobility Spectrometry (IMS) is frequently used to gauge the success of remediation in Clandestine Methamphetamine Laboratory (CML) sites. In most cases, remediation involves surface cleaning, followed by IMS interrogation to detect any residual contaminants. Even after seizure of the facility and with most contents removed from the structure, the remnants are still heavily contaminated with hazardous chemicals as well as methamphetamine. Besides direct chemical spill contaminations, airborne contaminants are absorbed or deposited onto surfaces throughout the facility such as the flooring, walls, ceilings, and structural supports.

The transport of methamphetamine in air and through surfaces is dependent upon the protonation state of the ionizable amine center on the molecule. While transport can occur as particulate, it can also occur in the vapor phase. The vapor pressure of methamphetamine as the free base (un-protonated) is 0.163mmHg, suggesting that methamphetamine will vaporize to a significant and detectable extent under typical indoor temperatures and pressures. Because it is an amine, detection limits for airborne methamphetamine are relatively low using IMS and as such, IMS is an ideal analytical tool to study the fate and transport of methamphetamine residuals left in remediated environments.

A Plexiglas chamber of size 1.5'x1.5' x 2' was made as airtight as possible (known to be watertight). The box offers the ability to place a hot plate within for heating, and a window along one wall in order to gain access to the plate. Along another wall there are three columns of four vertical ports. The first column contains an opening for IMS "sniffing," while the second allows for Solid-Phase Microextraction (SPME) to be accomplished through septa. The third column was added to track any temperature gradient that develops. A 4-channel datalogging thermometer was used for this purpose. The third wall of the chamber contains an opening 12"x12" allowing for a building material to be exposed on one side to vapors created within the chamber. This chamber establishes mock CML conditions into which building materials could be placed

For the analysis, methamphetamine salt was converted to its basic form and extracted into hexane. After drying, the methamphetamine was then gently heated in the presence of the building material being studied. The concentration of the airborne methamphetamine in the chamber was monitored at half-hour time intervals over 24 hours by IMS and every hour by SPME. Using both methods allowed for direct calibration of the IMS response for gas-phase methamphetamine. Chamber experiments were utilized to calculate diffusion coefficients for many different building materials. The ability to measure the concentration of gaseous methamphetamine in real time using IMS significantly simplifies the analytical process required to calculate diffusion coefficients and associated quantities such as flux. Experimental results were compared to those obtained with a simple modeling program and will be discussed.

Methamphetamine, Clandestine Lab, IMS

D10 Abuse of Synthetic Cannabinoid and Cannabimimitic Smoking Blends

Elizabeth M. Guest, PhD, and Jeffrey H. Comparin, BS, 22624 Dulles Summit Ct, Dulles, VA 20166*

After attending this presentation, attendees will understand the importance of laboratory analysis to determine the contents in the various smoking blends.

This presentation will impact the forensic community by showing that assumptions cannot be made as to the contents of any smoking blend, regardless of labeling.

The abuse of synthetic cannabinoid and cannabimimetic smoking blends is prevalent among teenagers and young adults who are seeking materials that emulate the effects of marijuana. These individuals often read internet blogs to determine which brand of smoking blend will result in the best euphoric feeling or "high." Users commonly make naïve assumptions concerning the active ingredient(s) and concentration based on the brand name or packaging of the smoking blends. In reality, the users do not know what compounds they are actually consuming. At present, there are at least two dozen different compounds that have been identified in smoking blends.

In addition, the manufacturing of these smoking blends is highly inconsistent. The compound is dissolved in a solvent such as acetone and either sprayed onto the plant material or mixed with the plant material in a mixer. The irregularity in these dosing processes leads to widely varying concentrations even within individual packages. Furthermore, many manufacturers often imitate the packaging of popular brands, thus leading to additional variability in the active compound and its concentration in blends with the same brand name. And finally, some blends contain more than one active component.

The Drug Enforcement Administration frequently receives law enforcement inquiries concerning a specific brand of synthetic cannabinoid smoking blend. These inquiries often stem from numerous local incidents or overdoses where a certain brand was used by the victims. Many hospitals are being flooded with abusers who overdosed on smoking blends. Medical personnel are handicapped when attempting to determine proper treatments because of the variability between samples.

This study investigated the concentration (percent by weight) of JWH-018 on dried plant material sampled within individual packages of smoking blend products across several popular brand names, including K2, Mr. Kwik-E, and Kush. The samples were first screened using gas chromatography-mass spectrometry (GC/MS) to determine if they contained JWH-018. A GC method was used to quantitate JWH-018 in each smoking blend. Cone and sampling techniques were used to investigate the concentration of JWH-018 in individual smoking blends of the same brand. These results confirmed the variability in the concentration of these compounds even within individual packages due to the dosing process. As a result, all other studies used plant material that was ground in a coffee grinder. The variability of the JWH-018 concentration between different packages of smoking blends with the same brand name and same packaging was then studied. Next, the concentration of JWH-018 was investigated across smoking blends with the same brand name but different labels (i.e., K2 blond and K2 melon). The concentration of JWH-018 was determined across smoking blends with the same brand name but different flavors (i.e., Florida Spice and Florida Spice Melon). Finally, the concentration of JWH-018 was compared in different smoking blends (i.e., Kush, K2, and Mr. Kwik-E).

This study proves that assumptions cannot be made as to the contents of any smoking blend, regardless of labeling. All such products require laboratory analysis to determine the contents in the various smoking blends.

JWH 018, Cannabinoid, Cannabimimitic

D11 An Investigation Into Volatile Organic Compounds That Have the Potential to Cause False Positives in Blood Alcohol Analysis

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After attending this presentation, attendees will understand how volatile organic compounds present in human blood can interfere with blood alcohol analysis, causing false positive results.

This presentation will impact the forensic science community by demonstrating the need for greater specificity in blood alcohol analysis.

The qualitative and quantitative analysis results of Volatile Organic Compounds (VOCs) in human blood and breath are used as biomarkers of human exposure for epidemiological monitoring and investigation, point of care testing for patient diagnosis and treatment, and as evidence in criminal cases in the forensic arena. Over 3,000 different VOCs have been identified in blood and breath samples of living people. These VOCs may be found in human blood and breath as a result of endogenous production or environmental exposure. Endogenous VOCs may be present as a result of normal metabolic activity, disease processes, or both. Exogenous VOCs detected in human blood and breath occur as a result of environmental exposure at the workplace, home, or other environments.

Some VOCs, generated as a result of normal metabolic activity, may have elevated due to metabolic diseases. For example, in diabetic patients, ketones and organic acids may be elevated in the blood during ketoacidosis. In addition, previous studies have identified key compounds, such as hydrocarbons, methylated hydrocarbons, sulfur containing compounds, nitrogen containing compounds, isoprene and acetone, as biomarkers for different systemic diseases. Also, research has shown that patients diagnosed with lung cancer, leukemia, bone cancer, and lymphoma have high levels of hexanal in their blood, while hexanal, 1-octen-3-ol, and octane were recognized as biomarkers for liver cancer. These VOCs have been detected in human blood using Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry (SPME-GC/MS).

Volatile organic compounds may be released to the environment by both natural and industrial sources. Compounds such as benzene, toluene, ethylbenzene and xylenes are present in fuel emissions. All of these compounds have been analyzed quantitatively in human blood using headspace SPME-GC/MS.

Ethanol is the most widely abused drug in the world. As a result, the most commonly known forensic application of VOC analysis is the determination of the concentration of ethanol in blood and breath samples from automobile drivers charged with Driving Under the Influence (DUI). The current standard for the determination of blood ethanol concentration is static headspace dual capillary column Gas Chromatography coupled to a Flame Ionization Detector (GC-FID). Other variations include single column (capillary or packed), direct injection, and GC/MS. If any compound present in blood were to co-elute with ethanol on the GC-FID, it would lead to a false positive result. A GC/MS method should replace GC-FID as the standard method for blood alcohol analysis as it would improve specificity and decrease the possibility of false positive results due to interfering volatile organic compounds. Many forensic laboratories have not budgeted for the upgrade to GC/MS for this type of analysis, and as a result, the GC-FID is still the most widely used analytical technique for blood alcohol analysis.

In this experiment, 46 VOCs that have been previously identified in human blood and breath were analyzed using GC-FID to determine if any of these compounds may co-elute with ethanol or the internal standard n-propanol, thus interfering with blood alcohol analysis. Of these 46 compounds, 17 co-eluted or had retention times sufficiently

similar to ethanol and n-propanol so that minor variations in stationary phase composition, flow rates or temperature would be expected to cause co-elution and thus interfere with blood alcohol analysis. These results demonstrate that many VOCs commonly present in the body can yield a false positive result for the analysis of alcohol in blood. Therefore, this work supports the idea that blood alcohol analysis is no different than blood drug analysis regarding the requirement to use the selective mass spectrometry detector for qualitative analysis instead of the non-selective FID detector.

Blood alcohol analysis should meet the same standards of scientific reliability as any other chemical test performed in the forensic toxicology laboratory.

Blood Alcohol Analysis, Gas Chromatography, Co-Elution

D12 Disinterments in the Desert: A Comprehensive Project to Identify the Missing and Unidentified Persons in Maricopa County, Arizona

Laura C. Fulginiti, PhD, John A. Piakis, DDS, and Christen Eggers, MS, OCME, 701 W Jefferson St, Phoenix, AZ 85007*

After attending the presentation, attendees will gain an understanding of the successful methods used to aid in the identification of unidentified remains that had been buried for more than 40 years.

This presentation will impact the forensic science community by demonstrating how a comprehensive approach to longstanding "Jane and John Does" can result in successful resolution, regardless of the length of time since death. The advancement of technology gives promise to the idea that it is possible to identify all of the deceased currently listed as unidentified. Every unidentified person is a missing person, and one of the responsibilities of the medical examiner is to identify the unidentified so that families and investigations can move forward.

The Maricopa County Medical Examiner's Office has approximately 230 unidentified persons dating back to 1979. The unidentified cases range from young children to adults. The causes of death range from natural to homicide. The Maricopa County Medical Examiner's Office received a federal grant from the Arizona Criminal Justice Commission (through NIJ) to exhume 50 unidentified persons in the hope that these individuals can be identified and notification to family can proceed. An exhumation team composed of medicolegal death investigators, forensic anthropologist, forensic odontologist, and funeral home representatives was formed. The main concentration of cases are cases from the 1970s through the 1990s when DNA technology was not as advanced as today so DNA samples were not collected and processed. Also, digital dental radiographs were not taken as that technology did not exist decades ago. The exhumations were performed at the Tempe Twin Buttes Cemetery in Tempe, Arizona, which is a Maricopa County indigent burial cemetery. Once the decedent is exhumed, the remains are moved to a portable examination table where the anthropologist examines the remains and collects samples for age estimation and ancestry. Specific demographics of the decedent's hair, length, type, and facial hair, etc. are noted so that a postmortem sketch can be created and posted on various missing and unidentified websites. The lead investigator documents all of the clothing, documents, and any individualizing characteristics. DNA samples are then collected by the lead investigator and the anthropologist; the DNA samples usually consist of clavicles, teeth, hair, and/or femur bone. The lead investigator submits the samples to the Arizona DPS Crime Lab where a profile is derived and entered into a federal DNA database. The odontologist conducts his examination using a portable digital dental radiography unit and laptop. A full mouth series of dental radiographs are completed and the radiographs are then automatically downloaded onto a laptop. Written documentation of the dental information is done, as well as dental charting. After the lead investigator confirms that the documentation is complete, all samples are collected, and the dental radiographs are complete, the decedent is placed into a new casket and returned to his/her respective gravesite. The lead investigator ultimately creates a written report of all the events during the exhumation and synthesizes the findings. The information is entered into national and local missing person/unidentified websites by the lead investigator who also contacts the law enforcement agency with jurisdiction to update them on the results.

A detailed explanation of the exhumation process and present summary data on the identifications will be presented. Nine scientific identifications have been made as a result of our efforts and there are solid leads on four other cases. Multidisciplinary efforts are coordinating into successful resolutions as the end goal is to make the identification and notify family. The presentation will point out that new technologies are always being developed and that the decision to cremate unidentified remains, a more common practice with some Medical Examiner Officers, eliminates the possibility of further examination in these important cases. Every unidentified person is a missing person and every person should be laid to rest with their name. While these cases are very challenging, they are solvable with appropriate resources.

Exhumations, Unidentified, Scientific ID

D13 Analysis of Three Special Cases of Paternity Testing

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After attending this presentation, attendees will have an understanding of paternity testing analysis and statistics analysis by probability of paternity.

This presentation will impact the forensic science community by validating the analysis of three special cases of paternity testing.

In Colombia, there are daily cases of paternity testing. Forensic genetics laboratory using the ICBF-INMLyCF Convention, have annually on average from 8,000 to 9,000 cases. These include cases with an alleged father, mother and minor reconstruction, cases with genetic profiles of the alleged father from the family, and case information from the exhumation of the remains of the alleged father.

In this paper discuss three special cases will be discussed one case in the determination of maternity and two cases of determination ofmpaternity.

Case 1 – Determination of Two Alleged Mothers: Which one is the biological mother of the child? Analysis was performed in 15 genetic systems and Amelogenin included in the Identifiler Kit Direct and found that the alleged mother 1 (PM1) was included as a birth mother; as to the Alleged Mother 2 (PM2), there was only found an exclusion of the 15 genetic systems. Since in order to report a real exclusion it requires at least three exclusions, we proceeded to the analysis of 13 additional genetic systems included in the kits from Promega PowerPlex CS7 and NGM Select Applied Biosystems, for 28 genetic systems. There were no more exclusions and, therefore, an opinion was issued in the two probabilities for the judge to determine maternity, as there was a degree of kinship between the two alleged mothers (mother and daughter).

Case 2 – Research Paternity Testing: The analysis of 15 genetic systems and Amelogenin included in the Identifiler Kit Direct was performed. Exclusions found two genetic systems and, therefore, an increasing number of genetic systems were analyzed, but found no additional exclusions. As the child was male, the analysis proceeded to chromosome "Y" and found two inconsistencies in the chromosome haplotype "Y." The question arises: How likely is it that the biological father presented four mutational events?

Case 3 – Research Paternity Testing: We performed the analysis of 15 genetic systems and Amelogenin included in the Identifiler Kit Direct. Three exclusions were found, but one of them was doubtful whether it was by the father or the mother. The number of genetic systems was increased to verify more exclusions (total of 28 genetic systems). As the child was male, testing proceeded with amplification of chromosome

haplotype "Y," finding an inconsistency with the alleged putative father. The question arises: Can a real catalog of paternity be excluded?

Paternity Testing, STRs, Statistic Analysis

D14 The Need for a Comprehensive and Interactive Digital Asset Management Software

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After attending this presentation, attendees will gain an understanding of the increasing amount and complexity of various types of data (digital assets) that are currently part of case evidence, and a corresponding method to contain, organize, and turn these digital assets into meaningful and retrievable information.

The impact of this presentation is to provide a meaningful method to turn the increasing load of data into information and improve access to its use as corporate information for QA, QC, teaching, training, and handling data in general.

A traditional digital asset management system (document management system) is a static, non-dynamic collection of data—an electronic filing cabinet. This type of repository solution may be adequate when used to specifically replace CDs, DVDs and disconnected hard drives as a method for data storage. However, while this solution may provide a level of security, organization, and easier access to the assets, it falls well short of what the real value could be, especially in the area of forensic science and the investigative services of law enforcement.

Many agencies traditionally just implement a simple data storage (repository) solution because of the overwhelming and the ever increasing amount and diversity of data. While this is justifiable, this is—not a progressive and powerful information producer—this approach will never allow these agencies to get beyond meeting the minimum level of digital asset management required by the regulatory and accreditation bodies.

Because the digital information created in the forensic world is not static, the multiple types of information that get generated need to support the investigative process. Therefore, digital asset/evidence management software needs to provide the tools to dynamically interact with the stored digital data and turn that data into valuable case information.

There is an increasing demand placed on every department to use these case-related digital assets in an attempt to answer the evolving questions about circumstances, cause of death, nature of evidence found, etc. Forensic agencies in general would greatly benefit from dynamically interacting with digital case information not only for themselves, but to be able to pass information on to other requesting agencies.

All of these agencies are gathering and developing an ever-growing amount of interrelated case evidence and digital image assets, audio/video files, HD data, federal and international data, and reports. To the degree that the communal change to dynamic digital asset management software is delayed, necessary training of individuals in the use and creative interaction with these powerful tools is withheld. Since all the departments ultimately work together, by default, then, the whole system is held back.

As the investigative tools are learned and the overall design is understood an individual who knows the basic concept of this type of software can easily migrate from one position/department to another. For example the adoption of a unified dynamic digital asset software package with an overall comprehensive and consistent design would not only provide useful and necessary skill for daily solution of case information but would become incredibly important and necessary in providing solutions in the chaotic aftermath of data collection and association in the event of a mass disaster.

As a solution to the above circumstances, a model will be demonstrated from the point of view of a medical examiners' office in the area of an analysis of a tool mark in biological material. This demonstration will take elements of the stored digital assets, perform the required analysis of stored images, and relate relevant investigative information and autopsy data to produce a report. Additionally, the report and all data used to inform the report will be associated in the relational database area of the software and allow for QA, QC of the report. This data and all other such stored case data represents not only stored case information but is accessible corporate knowledge that can be used for teaching, training, court room presentations, proficiency testing, and a host of other administrative uses. Additionally, such comprehensive and accessible data can facilitate the distribution to and interaction with other agencies which reduces the workload to distribute such required information.

Digital Assets, Tool Marks, Document Management

D15 A Case of Double Jeopardy: How 3D Laser Scanning Clarified the Truth

Thomas P. Mauriello, MFS, 8775 Teresa Ln, Laurel, MD 20723; and David A. Buerger, MS*, 1509 West St, Annapolis, MD 21401*

After attending this presentation, attendees will learn how 3D laser imaging can play a major role in determining characteristics and measurements of physical objects found in digital media and can "make the difference" in providing conclusive proof that in the past has been left to the subjectivity of art, rather than science.

This presentation will impact the forensic science community by bringing to their attention that having a client found "not guilty" alone may not influence the parole board's "a preponderance of the evidence" standard. Also, this case identifies an innovation in forensic image and video analysis in the area of object and facial comparison.

This is a criminal case involving first degree murder and arson, where the defendant was acquitted in 2007.[1] He was on parole for unrelated property crimes at the time of his arrest and acquittal for first degree murder and arson and therefore remanded to jail for revocation of his parole. The parole board has a different standard when determining the involvement of a parolee in a criminal act while on parole. If the parole board believes that a preponderance of the evidence suggests that the parolee committed the act in which they were charged, then the parole board can revoke the parolee's parole. This is exactly what happened in this case. Although he was completely exonerated for the murder/arson offenses, the parole board believed he was guilty, and therefore was returned to prison to serve out the rest of his unrelated 15-year sentence.

A parole revocation hearing was set and the defense attorneys contended that this was analogous to "double jeopardy," because the defendant was being retried to determine his innocence again. A forensic consultant was hired by the firm to evaluate all forensic evidence presented in the original criminal trial.

The prosecution's case relied heavily on surveillance video footage taken in the lobby of the high rise building in question. This presentation focuses on the surveillance video that shows a black man wearing a coat similar to the defendant, leaving the first-floor lobby stairwell during the time of the fire and murder. The crime scene was on the 10th floor. The investigators and prosecution contend even today that the subject in the surveillance video was the defendant leaving the scene of the crime.

The forensic consultant analyzed the video, consulted with facial recognition experts, and changed the contrast of the video in an attempt to clear up the image. He could only conclude that the person in question did not look like the defendant; he appeared to have a beard and mustache, unlike the defendant; and his build appeared to be shorter and stockier. This, together with other inconsistencies in the investigation, convinced the parole board at a parole revocation hearing and the defendant was freed from incarceration in 2008.

That should have been the end of the story, but the forensic consultant needed to know—"Was that the defendant in the stairwell or not?" The police investigator and prosecutor who tried this case are still adamant that it was. So he turned to the services of TransCon Imaging Solutions, who use 3D laser scanning to reconstruct vehicle accident scenes. In March of 2012, they used their 3D laser scanning equipment

to scan the entire lobby of the building in question, including the stairwell. Using their markers and measuring parameters, and superimposing the crime scene surveillance photos taken from the video footage over their scans, there is now conclusive evidence that the subject leaving the stairwell is 5-foot, 5-inches tall, and the defendant is 6-foot, 3-inches tall.

Reference:

1. State of Maryland v. Zukael T. Stephens - 2007

Double Jeopardy, Laser Scanning, Video Analysis

D16 The Hart House Report: A Multidisciplinary Discussion of Forensic Science in Canada

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After attending this presentation, attendees will understand the issues facing forensic science in Canada and will hear recommendations for improving deficiencies and evolving the science not only in Canada, but in local jurisdictions around the world where the legal system is questioning the reliability of forensic science.

This presentation will impact the forensic science community by providing a road map for forensic scientists in Canada and other jurisdictions to create and share advances in forensic science among academic and government practitioners. Forensic science must evolve to meet the questions and needs of the legal community on the reliability of forensic science and this presentation will offer some suggestions on how to accomplish that goal.

Over two days in May 2012, a group of forensic scientists and practitioners representing ten main disciplines within forensic science met at the University of Toronto, in a meeting sponsored by the Centre for Forensic Science and Medicine at the University of Toronto. The forensic scientists who participated were drawn from both the academic and public sectors and included the participation of a judge from the Ontario Court of Appeals.

A number of wrongful convictions and subsequent public inquiries in Canada have raised the question of reliability of forensic science in both the scientific and legal communities. This meeting was convened as an initial response to the question of reliability. There are three major trends driving change to forensic science in Canada:

1. A shift to an evidence-based paradigm, paralleling that in medicine.
2. Recognition among forensic science practitioners of the need to bridge the gap between expectations and deliverables in the presentation of expert opinion evidence.
3. The publication of the U.S. National Academy of Sciences Report.

The goals and objectives of the meeting were to:

1. Provide a description of the current state of forensic science in Canada.
2. Provide a summary of the major challenges and opportunities facing forensic science in Canada.
3. Provide recommendations on how to strengthen and develop forensic science in Canada.

Although the areas of forensic practice represented at the meeting were seemingly diverse (e.g., forensic nursing, forensic physical sciences, forensic psychiatry, amongst others), the group identified common deficiencies and adopted similar recommendations for the development and evolution of forensic science in Canada.

In the area of forensic science research, it was agreed that the ties between forensic science service providers (mostly government agencies) and universities had to be strengthened, with strategic and sustainable research programs being developed. In addition, it was agreed that national granting agencies should recognize forensic science as a discipline and apportion research funding accordingly. It was recognized that forensic science education and training would benefit from a more multidisciplinary approach and that graduate and postgraduate training should be developed and/or supported. Continuing education in best practices is necessary and this important aspect of forensic science needs to be developed more fully. In order to do this, scientific working groups and their guidelines, professional certification and accreditation and recognizing the importance of ethics, professionalism and bias will need to be instilled in practitioners. An in-depth discussion of the administration and regulation required to do this was also undertaken.

The meeting concluded by recognizing that forensic science is an integrative, multi-disciplinary science with its own theoretical basis requiring scientific inquiry. In order for forensic science to strengthen its progress in Canada, it is vital that all areas embrace the full cycle of service, teaching, and research. While the group recognized that the discussion was only introductory, a substantive document was produced which should assist in addressing these deficiencies and attaining these goals. The document does not recommend the creation of new agencies, nor does it advocate for specific systemic reforms, rather it is offered as a tool to engage stakeholders and encourage further dialog so that forensic science can continue to evolve, grow, and meet the needs of the legal system.

Forensic Science, Canada, Reliability

D17 A Practical Guide to Processing Synthetic Cannabinoid and Cathinone Operations

Jay R. Hopenwasser, MS, 10150 E Technology Blvd, Dallas, TX 75220*

After attending this presentation, attendees will gain the necessary knowledge to safely access and process an illicit operation that manufactures synthetic cannabinoid and cathinone commercial products.

This presentation will impact the forensic science community by providing an overview of the inside operations of a processing site for the manufacturing of commercial and illicit “spice” and “bath” products, recognizing the dangers with these processing sites and how to safely collect evidence.

The emergence of synthetic cannabinoids and cathinones in the illicit drug market has introduced new challenges to law enforcement and their forensic laboratories. Law enforcement has been raiding retail operations which deal in “spice” and “bath salt” products, but are now encountering processing sites that are responsible for manufacturing these commercial and illicit products. These processing sites may be unfamiliar territory for forensic chemists and law enforcement officers. These processing sites present new challenges due to their differences from a typical clandestine methamphetamine laboratory.

Respondents would be at an advantage knowing what to expect to find at these illicit operations where different chemicals, apparatuses, and potential dangers will be encountered. Furthermore, forensic laboratory and law enforcement personnel must be familiar with processes such as the preparation and application of raw powders and flavorings to plant material. This presentation will provide an overview of these synthetic compound processing sites. Furthermore, the impact of the recent federal scheduling and analogue classifications of these compounds with respect to the processing of these sites will be discussed. Participants will acquire the necessary knowledge to approach, process, and analyze these new illicit laboratories.

The sheer volume of evidence at these processing sites could be overwhelming for law enforcement. Importantly, laboratory personnel must know how to sort through the bulk material for the important items needed for evidence. Challenges that one might face while processing the site will be presented. Since these sites often contain large amounts of suspected drug substances and thousands of packaged items, ways to process the site will also be discussed.

The analysis of synthetic cannabinoids and cathinones can present challenges to the forensic chemist. The continued introduction of new synthetic compounds results in forensic chemists repeatedly encountering unknown spectra. For instance, the active drug ingredient in synthetic cannabinoid samples has changed from JWH-018 to AM-2201 and recently to UR-144 and XLR-11. Matters are further complicated with the addition of synthesis byproducts and isomers (i.e., 3-methylmethcathinone vs. 4-methylmethcathinone) of these unknown compounds. At times, sample preparation to remove impurities becomes crucial to obtain clean spectra for the verified identifications. This presentation will also provide simple steps and tips for the forensic chemist to use in the analysis and identification of these synthetic compounds.

Synthetic, Cathinone, Cannabinoid

D18 The Italian Killer of JFK: History and Evolution of Carcano Model 1891 — An Italian Bolt-Action Military Rifle

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After attending this presentation, attendees will understand the history of the rifle used in the JFK assassination, the Carcano Model 1891, an Italian bolt-action military rifle based on the introduction of the original 1881 model that continued production through World War II.

This presentation will impact the forensic science community by illustrating a different approach to the historical analysis of firearms.

Perhaps Oswald was not aware of the piece of history he had in his hands when he bought the weapon for the assassination attempt on President Kennedy; he based his choice on economic reasons, but that Carcano 91/38 rifle was the result of a long evolution of the main Italian service rifle during the two World conflicts.

This study proposes an anthropological approach similar to an evolutionary phylogeny of this weapon, attempting to illustrate the pressures that generated the different models which had been employed in the main war environments of the twentieth century.

The common ancestor of the Carcano Model 1891 was the Vetterli-Vitali 1870/90. The Carcano was developed in 1891 from this previous weapon after the invention of smokeless powder in 1884 and after the adoption of the bolt-action rifle Steyr-Mannlicher M1895 by the Austro-Hungarian army and the Mauser Kommission by the German army.

The Carcano rifle presents two divided branches of its "phylogenetic tree:" the first was the production of a short weapon (Moschetto Model 1891) mainly designated for the Cavalry Division, the Royal Carabinieri; and, the Bersaglieri light infantry, while the second would create the line that led to Oswald's weapon.

The substantial difference between these two models, bolt-rifle and musket, is the dimensional reduction of the second one with respect to the rifle, to the barrel, and of the bayonet cost, entirely motivated by the need to provide the high-mobility of the infantry units with an efficient, light, and short weapon.

World War I introduced an upset in European structure and dynamics, and the Carcano Model 1891 had to adapt to the changes of the forthcoming conflict. No longer fossilized in trench warfare, it had to become a rather active tool in the more diffuse mechanization of the armies. This gave birth to the short Carcano Model 1891/38, a lighter and more manageable weapon that partly seems to inherit some of the musket's characteristics. In contrast to the biological evolution that does not seem to step backward, this rifle took place a return to the caliber used in previous models (6.5 x 52mm; .268cal); this "involution" was not due to technical needs, but was realized for economic reasons: the Staff Officers wanted to recycle the surplus of old ammunitions from the first world conflict. This Model 1891/38, with strengthening of the range, made history because it fired the fatal shot to President Kennedy, but it was also the protagonist of numerous battles and action in WWII.

Nonetheless, this model does not represent the last evolutionary phase of the Carcano rifle, which is expressed by another descendent that seems again to look back upon the past. The Carcano Model 1891/41 turns out to be bigger and more cumbersome; however, it can reach an excellent performance in ballistic shooting. The extinction of the Carcano rifle as the main service weapon for the Italian Army is historically represented by the end of World War II, when a new "population" of weapons was introduced by the Allied troops.

This study will also propose to the scientific community a new evolutionary approach to reconstruct the history of a specific weapon's model, also presenting historical aspects of a very infamous rifle often left in the background of the President Kennedy assassination.

Military History, President Kennedy, Ancient Weapon

D19 Antique Firearm Examined With Scanning Electron Microscope (SEM) to Determine the Persistence of Firearms Discharge Residue (FDR)

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After attending this presentation, attendees will understand some of the developmental features and characteristics of the Smith Carbine, the type of components used in the manufacture of 19th-century ammunition, and the identification and persistence of Firearms Discharge Residue (FDR) in an antique firearm.

This presentation will impact the forensic science community by providing an in-depth Scanning Electron Microscope (SEM) examination to determine the persistence of firearm discharge residue from an 1876 Smith Carbine. The techniques used in this examination will determine if FDR remains in the bore of the barrel after a period of three decades.

The Smith Carbine fired by Henry Mason Wheeler during the Northfield Bank raid on September 7, 1876, was examined for FDR. Even though antique firearms are collected for aesthetic reasons, many continue to function as a firearm and could be used in the commission of a crime. However, limited research has been conducted on the persistence of FDR in antique firearms.

In this study, the Smith Carbine had not been fired in over 32 years. Since the 1876 raid, only six other individuals have owned the Smith Carbine other than Edward Dampier, the original owner. The six owners include: Dr. Charles Dampier, Henry Mason Wheeler, Mae McCulloch Wheeler, Henry Mason "Hank" Wheeler, Jr., Charles R. Dickson, and the present owner in Grand Forks, North Dakota, who has requested to remain anonymous.

The Smith Carbine was developed by Gilbert Smith of Buttermilk Falls, New York. Smith developed a method for sealing the chamber in a breech-loading gun and was granted patent # 15,496 on August 5, 1856. A series of patents led to the development of a carbine identified as the Smith Carbine. The carbine originally fired a manufactured paper cartridge but subsequently fired manufactured cartridges made from other materials. The actual number of Smith Carbines produced is unknown; however, the military purchased approximately 30 thousand carbines and approximately 19 million cartridges. The Smith Carbine was primarily used by the Union Cavalry during the Civil War.

The firearm examined was a single-shot breech-loading .50 caliber Smith Carbine and serial number 19359 was stamped near the hinge. A sample was collected from the barrel using a 100% cotton patch. Residue from the cotton patch was collected using two aluminum stubs with adhesive applied to the end of the stubs for SEM analysis. The sampling procedure involved contacting the adhesive end of the aluminum stub on the surface multiple times to collect the FDR.

In the early 1800s, a typical percussion cap primer mixture contained potassium chlorate, charcoal and sulfur. However, by the time the Smith Carbine was developed, the percussion cap primer mixture consisted of mercury fulminate, potassium chlorate, antimony sulfide,

and powdered glass. The metal cup that contained the priming mixture was made from copper or brass. A typical formula for black powder during the Civil War era would have consisted of 75% potassium nitrate, 15% charcoal, and 10% sulfur. Early black powder formulations substituted sodium nitrate in place of potassium nitrate; however, the powder was considered inferior because the sodium nitrate absorbed moisture. A typical alloy cast bullet contained 90% lead, 5% antimony, and 5% tin.

The type of electron microscope used in the analysis was a JEOL 5800 Low Vacuum Scanning Electron Microscope (LV SEM). The operating conditions of the SEM included an accelerating voltage of 25keV with a beam current of 0.6nA. Analyses were performed in the low-vacuum (p = 17 Pa) mode of the SEM. A working distance of 11mm was used for the examinations.

Sixteen particles were analyzed on stub #1 and 23 particles were analyzed on stub #2 to produce an elemental profile from the barrel residue. The particle sizes were found to be approximately 1µm. The Energy Dispersive Spectrometry (EDS) analysis from the residue revealed the presence of ten elements. They included antimony, barium, calcium, copper, iron, lead, potassium, silicon, sulfur, and tin. However, one element not detected was mercury.

Seven of the ten elements collected and analyzed (antimony, copper, lead, potassium, silicon, sulfur, and tin) are consistent with 19th-century ammunition components. The silicon likely originated from the powdered glass portion of the priming mixture. Since iron is not an ammunition component, this metal could possibly derive from the barrel of the carbine. The source for the barium and calcium are unknown. However, these two elements could be contaminants in the ammunition or they could have been introduced into the barrel as a result of swabbing the bore during the cleaning process.

In conclusion, the SEM examination demonstrates that FDR can persist in the bore of an antique firearm for more than three decades. The SEM did not detect any particles of mercury in the residue. Most mercury particles vaporize when the firearm is discharged and the remaining mercury particles may dissipate since mercury is a volatile element. Although it is difficult to estimate the time of discharge, absence of mercury could indicate the weapon has not been fired for an extended period of time.

SEM, Firearms Residue, Antique Firearm

D20 Soft Classification by Combined Target Factor Analysis and Bayesian Decision Theory

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The goal of this presentation is to inform attendees of a new chemometric soft classification method that can be applied to forensic problems.

This presentation will impact the forensic science community by providing information on a soft classification method that is designed to work in the presence of unspecified background interferences and provide the user with probabilities of correct class assignment.

There are analytical problems in forensic science where individualization is not currently possible; however, the analyst may benefit from assigning an analyte to one or more possible classes. This is especially true for mass produced items that do not involve DNA analysis. When analysis of the item of interest is free from significant background interference, existing classification methods (i.e., linear discriminant analysis, partial least squares discriminant analysis, etc.) may be applicable. However, when there is significant background interference, classical discriminant analysis methods may fail, especially when the background signature is unspecified and possibly unknown. Fire debris analysis is one example of this type of problem. The analyst's goal is to determine if ignitable liquid residue is present and, if so, assign the residue to a class under the American Society of Testing and Materials (ASTM) Standard Method E1618.[1] Pyrolysis product contributions may be large and by coincidence, some pyrolysis products may be the same as ignitable liquid components. In these cases, visual pattern recognition will become more challenging as the ignitable liquid signature decreases and the background signature increases. A new method of addressing this problem is examined by combining Bayesian soft classification with target factor analysis (TFA). The method will be described and test results from the analysis of fire debris data will be presented.

The method relies on analysis of the average mass spectrum across the chromatographic profile (i.e., the total ion spectrum, TIS) from multiple samples collected from a single fire scene.[2] The multiple TIS are concatenated into a single data matrix and subjected to abstract factor analysis, and target factor rotation. TIS from reference ignitable liquids with assigned ASTM classifications are used as the target factors in TFA. Class-conditional distributions of the correlations between the target and predicted TIS spectra for each ASTM class are represented by kernel functions and analyzed by Bayesian decision theory.[3] The soft classification approach assists in assessing the probability that ignitable liquid residue from a specific ASTM E1618 class is present in a set of samples from a single fire scene, even in the presence of unspecified background contributions from pyrolysis products.[3,4]

The method was tested on: (1) computationally generated data sets; (2) small-scale laboratory burn experiments; and, (3) large-scale experimental burns. In all cases, the correct classification rates exceeded 80%. The method and results will be discussed along with the potential for expanding the method to other forensic applications.

This project was supported by Award No.2008-DN-BX-K069, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The project involved collaboration with the Bureau of Fire Standards and Training, Ocala, FL, and the Bureau of Forensic Fire and Explosives Analysis, Tallahassee, FL. 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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Fire Debris, Soft Classification, Factor Analysis

D21 Validation of Semen Detection Kits for Field Application in Sexual Assault Cases

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After attending this presentation, attendees will have a general understanding of the sensitivity and specificity of four semen detection tests, two crime-scene-ready-kits and two used primarily in a laboratory setting. This study utilized four substrates, three US Air Force uniform fabrics and a cotton fabric, which allowed determination of semen behavior and biofluid ability when applied to these substrates.

This presentation will impact the forensic science community by indicating whether crime-scene-ready semen detection kits are comparable to laboratory kits in sensitivity and specificity, and whether or not a crime-scene-ready kit should be implemented for field application, in particular by Department of Defense (DoD) criminal investigators. Conducting such tests on certain items of evidence may possibly decrease the amount of backlog in the servicing forensic

laboratory, as investigators may be able to better identify items of evidentiary value for laboratory submission.

Synopsis: Currently, DoD, specifically United States Air Force, criminal investigators conduct presumptive tests for certain biofluids; however, field-testing for the detection of semen is not conducted at crime scenes. There are a number of concerns associated with conducting these tests at crime scenes, which include sample consumption, as well as potential erroneous test results. However, semen detection tests could also be extremely beneficial for a number of reasons. In military sexual assault cases, conducting a semen detection test could not only help corroborate a victim's statement, but also provide probable cause for a search warrant, which could garnish additional probative evidence.

In order to determine which crime scene-scene-ready kit, if any, should be used at these crime scenes, the following tests were validated: ABAcard p30 for Crime Scene, ABAcard p30 for Laboratory, Rapid Stain Identification (RSID™) – Semen, and the Serological Research Institute Acid Phosphatase (AP) Spot Test. Again, four different fabrics, used to manufacture uniforms and/or often associated with sexual assault cases, were utilized in the study. To test the sensitivity of each test, a neat sample and four dilutions (1:10, 1:100, 1:500, and 1:1000) were prepared; fifty microlitres of neat or diluted sample were applied to each substrate, and tested. Furthermore, the fabrics that provided positive results were washed, either once or twice, to determine if semen was still detectable. A volume sensitivity test was also conducted to further determine the sensitivity of each test. To test the specificity, three chemically similar biological fluids were also applied to each substrate and tested. Nine lubricants/pseudo lubricants often seen in sexual assault cases were also tested (mixed with/without semen) to determine if these interfered with the semen detection or provide a false positive result when no semen was present in the sample.

Results/Conclusion: All tests were positive when the samples were neat; however, results differed when the samples were diluted. In the washing stage of the study, the RSID™ – Semen performed the best, detecting semen on two of the four fabrics after these were swabbed a total of three times, and washed twice. The AP Spot Test performed the best in the volume sensitivity test; however, when mixed with one of the nine lubricants, the AP Spot Test failed to detect the presence of semen. These same results were obtained with the ABAcard p30 for Crime Scene kit, also with the same lubricant. All tests provided negative results when urine and saliva samples were used; however, the RSID™ – Semen provided a false positive for fecal matter.

Based on the tests conducted and observations made in this study, between the two crime-scene-ready kits, the RSID™ – Semen produced, overall, better results. A recommendation to implement the RSID™ – Semen kit for field application by DoD criminal investigators has been made.

The opinions or assertions constrained 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.

Semen Detection, RSID, Crime Scene

D22 Behavioral Evidence: Following the Traces of a Disappearance in a Case Study

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After attending this presentation, attendees will learn about the first case convicted in Colombia with behavioral evidence, and see how the criminal behavior special unit of the attorney general's office carried out analysis of a mysterious disappearance of a woman, the wife of a prestigious doctor.

This presentation will impact the forensic science community by showing the dramatic effect of behavioral analysis in a case without any physical evidence to reach a verdict in the Colombian criminal justice system.

On August 8, 2007 Luz Amanda Vargas Lemus disappeared and lost contact with her family. Her children were the last people who saw her when they went to school that day. With this information, a Missing Persons Report immediately activated the search; however, this did not provide information to her whereabouts, because her cell phone was off. After the report investigators contacted Jesus Guillermo Burgos Bacca, the victim's husband, who then disappeared without a trace and had left his job in the hospital where he was a gynecologist. Immediately the criminal behavior analysts were called and with the information obtained through interviews, the team was able to assess the victimology of the woman. She was a sociable person, emotionally expressive, but had aggressive behavioral manifestations including jealous behavior. With her two sons, she had a protective and responsible parental behavior.

The house of this family showed no signs of violence within the residence; however, the crime scene investigators apparently found blood stains. The investigators applied a latent blood reagent, which reacted showing different patterns in various parts of the house. All spots were sampled and the laboratory obtained a DNA profile that was consistent with the genetic profile of Luz Amanda. The analyst group was surprised to find no blood from Burgos, but his belongings were found, while his wife's belongings were not.

During the investigation, it was found that the couple had a dysfunctional relationship with an escalation of violence. It was very interesting for the investigation team that they found the blood of only one of them at the scene in a distribution pattern that went from the bathroom to the garage of the house. Jesus Guillermo was the only person for which there was no information about his whereabouts on August 8; he had exhibited different behavior than usual for the days before this event, including the purchase of a gun, massive withdrawals of money, and changes in work schedules in hospital. This continued with a variant in behavior culminating in his disappearance, leaving his personal and professional life. His flight intentions remained unknown until the time of his capture when he was very close to leaving the country using false documents.

Jesus Guillermo was put on trial for the crime of forced disappearance. After four weeks of hearings in which the prosecution and defense presented their evidence, the judge found him guilty based on behavioral evidence presented that showed pre-meditation and post-offense conduct. Currently he is paying a sentence of 32 years in prison. To this day the body of Luz Amanda Vargas Lemus has not been found.

Behavioral Evidence, Forced Disappearance, Criminal Behavior

D23 Forensic Pathologist, Forensic Entomologist, and Medicolegal Death Investigator: Forensic Team Approach to Death Investigation

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After attending this presentation, attendees will understand the role of the forensic pathologist, entomologist, and death investigator in determining the time of death, the cause of death, and the manner of death, also increasing the awareness of the forensic community regarding coordination of multiple forensic disciplines in death investigations.

This presentation will impact the forensic science community by showing the forensic team's value to a medical examiner/coroner's office in the investigation process, criminal trial, and for final closure for the victim's families. It also aims to allow for an organized approach to help determine if the death is criminal in nature or if the death is a result of other non-criminal circumstances.

An 18-year-old Caucasian female reportedly had been missing one week prior to being found in a remote area in a field. Reportedly, she was last seen abducted from her home by three males and forced into a

vehicle. She was found clad, in a grassy area in an open field with apparent blunt force injuries of her head, neck, and abdomen with decomposition and early maggot infestation. Autopsy findings included blunt force head injuries, multiple stab wounds, defensive injuries of the hands, asphyxia due to smothering or strangulation, and postmortem changes with insect activity and decomposition. Toxicology results were positive for cocaine. The cause of death was multiple blunt and sharp force injuries and the manner of death ruled as homicide.

This presentation will increase the awareness of the forensic community and law enforcement agencies in the attempt to show, how the forensic professionals can work together in the forensic autopsy. The focus of this case presentation will highlight the forensic team approach. The difficulty of the case stems from advanced decomposition of the body, determination of the cause and manner of death, the history behind the investigation, and the final court decision. The presentation will include the role of the medicolegal death investigator to collect insects found at the scene of a decomposed body, how the forensic pathologist determines the cause and manner of death, and how the forensic entomologist determines the time of death.

The role of the Medical Death Investigator (MDI) is to communicate with police, witnesses, and the family of the deceased, and to make observations and documentation through photography, diagrams, and written reports. The MDI conducts a preliminary examination of the body to establish postmortem interval (PMI) and discover injuries. In cases of decomposition, knowledge of collection, preparation, and packaging of insects is vital in assisting the forensic entomologist in making a determination of time of death. The forensic pathologists can make a determination of the cause and manner of death through the forensic autopsy following the forensic autopsy standards established by the National Association of Medical Examiners (NAME). In decomposed bodies, it is more difficult to recognize injury patterns, assess ante-mortem from postmortem injuries, and establish positive identification. The forensic entomologist has special knowledge regarding insect succession patterns in decomposing human remains and provides information regarding time of death and possible location of death. Certain insects will lay eggs on dead bodies. Most insects exhibit predictable life cycles. By observing the development stage of insects found on a body, and by taking certain environmental factors into account, a postmortem interval can be estimated.

In conclusion, the abilities of the forensic team approach, including the forensic pathologist, forensic entomologist, and the medicolegal death investigation, can be invaluable to a medical examiner/coroner office in the investigation process, criminal trial, and for final closure for the victim's families.

Pathology, Forensic Entomology, Scene Investigation

D24 Forensic Archaeology and the Good Friday Agreement: The Search for the Disappeared in Ireland

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After attending this presentation, attendees will learn about the work done by the Independent Commission for the Location of Victims Remains (ICVLR) in Ireland and the specific context in which it operates and discuss the need to reassess traditional search methods to increase successful search probability.

This presentation will impact the forensic science community by illustrating the need of forensic archaeology to improve upon its theories and methods, also demonstrating the manner and means to provide a more efficient search service.

The ICLVR was set up in 1999 by legislation that derived from the Good Friday Agreement (GFA). The GFA was the legislative turning point in the solution to the political conflict that had been escalating in Northern Ireland since the late 1960s. This Agreement set out to design a system in which Northern Ireland could move away from political violence and evolve gradually toward a peaceful environment based on mutual respect. It was deemed necessary by the GFA for this reconciliation process that the governments of Ireland and Northern Ireland commit to locating the remains of victims who had "disappeared" during a particular period of paramilitary activity. The ICLVR legislation allowed for the search for victims that fall into the category and time frame described by the legislation. It specifically excluded the forensic investigation of the "recovery" sites and the use of any such information for a criminal prosecution. To date 16 people fall into the category covered by this legislation and at the time of writing, nine of them have been recovered. Currently these cases that fall into the specific legislative remit range from 1972 to 1985 and present a unique and complicated challenge to those involved in their search and location.

At the outset, search activity was delegated to local law enforcement and following an initial 30 days of intense work search, was scaled down and took place on an intermittent basis over three years. Following calls for outside expertise, an independent forensic expert was engaged in 2005 to advise on how best to proceed. This marked a new phase and a new period of fieldwork activity that began in earnest in 2007. Search activity was extended to include the employment of forensic archaeology methodology. There followed a concentrated period of search over the next five years by a team of specialist independent advisors. This paper will look at the case specifics in terms of site type and search methods employed from the perspective of the forensic archaeologist and will look at the implications that follow for those searching for victim remains in other locations and political contexts.

Thus this paper will look at the forensic methods that were used and their relationship with the site type. The search methods considered and employed will be discussed in terms of merit and usefulness in light of the requirements of the search for "the disappeared." It will also consider how direct interaction with victims' families influenced the process and how initial identification of the scenes was based on confidential information received from those involved in the original killings.

The paper will demonstrate how the accuracy and specificity of the search for buried remains can be improved by the constant reassessment and reconsideration of the methods to be deployed. This will lead to more efficient searches of a potential body recovery location.

Forensic Archaeology, Search, ICLVR

D25 Managing Mass Fatality Incident Response: Concept of Operations and Field Operating Guides for an Integrated Regional Response to Mass Fatality Incidents

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After this presentation, attendees will: (1) learn the essential components of a regionally integrated concept of operations for mass fatality incident response that reconciles variable asset availability associated with large and small jurisdictions; (2) gain a better understanding of the division of responsibilities at the incident site, morgue, and Family Assistance Center; (3) gain a better appreciation for Mass Fatality Incident (MFI) response strategies that may be applied to any type of disaster; and, (4) learn about the utility of field operating guides for providing real-time guidance to MFI responders.

This presentation will impact the forensic science community by providing guidelines for a cross-jurisdictional, multidisciplinary, collaborative response to mass-fatality incident management.

The Houston-Galveston Area Council (H-GAC) Region consists of the City of Houston and 13 surrounding counties. The Department of Homeland Security (DHS) designated the H-GAC Region as one of seven high-risk urban areas in the United States for potential catastrophic incidents and subsequently awarded grant funding for regional catastrophic response planning. In late 2010, a mass fatality

incident (MFI) planning team was assembled to develop response strategies for the H-GAC Region.

The MFI response planners developed and published a Mass Fatality Management Concept of Operation (CONOPS) for the 13 county H-GAC Region. The plan offers an integrated plan for unified response to catastrophic incidents producing mass fatalities that overwhelm local resources. This CONOPS complies with the Incident Command System and the National Incident Management System. It is designed to be flexible and scalable, and addresses four separate components of incident response: site management, morgue operations, victim identification, and Family Assistance Centers (FAC). It also recognizes that incident response starts and ends locally and external regional, state, and federal resources serve in support roles for the affected jurisdiction(s). The plan also leverages existing non-MFI related local and regional capabilities to fill MFI-related response gaps.

To develop this CONOPS, the team formed several advisory groups to assist in guiding the process. Eight specific groups were assembled: logistics, information technology, investigations, fatality management and identification, legal issues, family assistance, hazardous materials, and pan integration. Group participants (five to ten per group) represented a majority of the 13 counties within the region. Stakeholders were included from county and municipal government; federal, state, and jurisdictional agencies; private industry; non-profit organizations; and non-governmental agencies. The benefits achieved through use of advisory groups were: engaging stakeholders in the planning process, drawing from their expertise, and subsequently achieving stakeholder buy-in of the final product.

At the beginning of the project, a kickoff meeting was held for potential regional stakeholders and, after presenting a project overview, volunteers were solicited for membership to the various advisory groups. Then, specific subject matter expertise was sought out from other agencies, organizations, and jurisdictions to round out regional representation. Convening group meetings helped to identify specific concerns, develop integration strategies, and build working relationships across disciplines and the region. The advisory groups will be sustained to maintain MFI awareness in the region and to inform both future MFI responses and planning efforts.

Contents of the CONOPS included a hazard and vulnerabilities assessment, capabilities assessment, assumption statements, communications section, logistics section, various operational components, and appendices. Specific responsibilities are delineated in an appendix of Field Operating Guides (FOGs).

The FOGs are built from a comprehensive organization chart that reflects the various components necessary for an MFI response. The 52 individual FOGs are design-based from the NDMS DMORT response model. Each guide includes a mission statement; description of required knowledge, skills, and abilities; general responsibilities; and associated key tasks. The FOGs are divided into major divisions of each response element—site, morgue, victim identification, and FAC. Site management includes human remains recovery, personal effects, and human remains transport. Morgue operations are divided into admissions and processing, and forensics units. The Victim Identification Center contains sections for postmortem data collection and management, data analysis, and quality assurance. The Family Assistance Center consists of a forensic unit, family management unit, and health and human services unit.

The completed CONOPS is used to help guide the training and exercising of disaster response across the H-GAC Region. The planning initiative was funded by DHS and delivered end products are available for distribution upon request.

Fatality Management, Concept of Operation, Field Operating Guides

D26 Excavation of Mass Graves in Cyprus

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After attending this presentation, attendees will learn about mass graves in Cyprus and the techniques that are used to exhume human remains.

This presentation will impact the forensic science community by providing examples of practical methods and techniques used by the CMP bi-communal forensic team in the excavation of mass graves in Cyprus.

In 1981, talks between Turkish Cypriot and Greek Cypriot leaders were held under the auspices of the United Nations which led to an agreement for the establishment of the Committee on Missing Persons in Cyprus (CMP). The CMP is mandated to establish the fate of missing persons without investigating cause or establishing accountability. The number of missing persons from both communities stands at 1,958, with 1,464 Greek Cypriots and 494 Turkish Cypriots missing. On August 28, 2006, the CMP formalized the beginning of a bi-communal program of exhumation across the entire island.

Most mass graves excavated in Cyprus are in open fields, pits, wells, caves, hills, mountains, stream and river beds, with open fields and well excavations being the most common type of mass grave encountered in Cyprus. Despite these commonalities, once the excavations have started, methods may be modified to take into account the unique characteristics of each site as they are presented. This presentation contains examples from all the mass graves that have been exhumed by the CMP Bi-Communal Forensic Team (BCFT).

Mass graves are a complex and confusing mix of bodies, body parts, grave fill, and artifacts. A mass grave can contain complete bodies in layers or partial bodies—commingled and complete bodies in a single burial feature. The exhumation techniques focus on detailed recording; using photography, sketching, site mapping, and categorizing the recovered remains as bodies, body parts, and general body parts, which may be associated with recovered artifacts.

When mass grave burial features are encountered for the first time, the excavation proceeds in a manner to define the horizontal and vertical limits of the site grave feature and to expose the evidence *in situ*. This is particularly the case with sites under threat from development and damage. In these cases, a combination of methods is used.

After setting the boundaries of the mass grave, a bit larger than the real margin of the grave, the soil from the burial feature is removed manually and systematically. Once remains and artifacts are discovered, further removal of soil is performed by using small masonry scoops and wooden sticks. The associated grave fill is sieved in order to recover small bone fragments and unassociated teeth.

Before removing the uncovered remains, all paper bags, boxes, and forms are properly labeled with the appropriate code to ensure that provenience and context is maintained. Taking detailed photographs together with the appropriate documentation during the critical points of cleaning and lifting each skeletal unit, complete or partial skeleton, has proven essential. Placing flags in the areas where human remains have been located facilitates subsequent mapping with a total station.

At the conclusion of the fieldwork, all recovered evidence is handed over under chain of custody to the CMP Anthropology Laboratory and the BCFT prepares an excavation report describing context and associations of recovered evidence. The archaeological data and reports, when combined with the skeletal and genetic reports, form the basis of nearly all CMP identifications.

Mass Grave, Excavations, Techniques

D27 The Persistence of Forensic Soil Evidence on Denim and Car Seat Covers: Getting the Dirt on a Suspect

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After attending this presentation, attendees will better understand the post-transfer behavior of forensic soil evidence. How forensic soil evidence acts during the transition from primary transfer to secondary and higher transfer will be discussed, as well as how the secondary sample of soil compares to the original sample. How various fabrics, specifically car seat covers, play a role in the post-transfer behavior of soil will be explored. Attendees will also have a better understanding of the difference between primary and secondary transfer. An explanation of why this information could be useful to law enforcement and crime scene investigators will be discussed. Finally, recommendations for forensic and crime scene investigators regarding the collection of forensic soil evidence, to include soil-covered clothing, will be suggested and discussed.

This presentation will impact the forensic science community by providing understanding that both fabric weave and fabric type affects the transfer and post-transfer behavior of soil. This information will be useful to forensic and crime scene investigators. When coupled with all other evidence relevant to the case, it can assist investigators in linking persons involved with the scene of a crime.

A study of the persistence of forensic soil evidence was conducted on a variety of garments and fabrics including car seat covers. Car seat covers and denim pants with different types of fiber composition and fabric weave were used. Dry soil was rapidly lost from cotton pants with a stain-resistant backing, and persisted on cotton denim pants—through walking around and then by driving a vehicle. Soil deposited with a wide range of particle sizes initially persisted through primary transfer onto denim fabric throughout secondary transfer to a car seat. Clean denim was then used to determine the post-transfer behavior of the soil.

In some trials, the secondary and higher transfer soil evidence survived motion and activity and could be examined microscopically, whereas in other trials it could not. The smaller fraction of forensic soil evidence deposits can be detected after secondary and higher transfer has occurred, even well after larger particles have been lost from the first transfer surface. This demonstrated the selective loss of larger grain particles that is often suspected in cases submitted to the crime lab.

One immediate observation made was that some soil deposits are extremely difficult to see, especially on darker colored fabrics. The research showed that both fabric weave and fabric type affects the transfer and post-transfer behavior of forensic soil evidence. The size and texture of the material being transferred may affect the persistence of trace materials, which is linked to the type of surface on which the material is being retained. This research showed that a cotton denim weave was a nice retainer for small and very small soil particles. A significant difference between primary, secondary, and higher transfer of soil was found, when compared to the exemplar sample. It was determined this was due to gravity, along with any subsequent movement made by persons involved at a crime scene. This research showed it was apparent that large soil particles were rapidly lost after the initial transfer from ground to denim. Finally, this information is found to be useful to forensic and crime scene investigators, when coupled with all other evidence relevant to the case, as it can assist investigators in linking persons involved with the scene of a crime and/or with a victim.

Forensic Evidence, Soil Evidence, Soil Particles

D28 Assessment of Portable Forensic Detection Equipment and Methodologies on Biological Evidence From Commonly Encountered Forensic Substrates

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After attending this presentation, attendees will be familiar with multiple forensic detection methods that are portable and applicable for field forensic operation, offering effectiveness at detecting biological samples from commonly encountered forensic substrates, with consideration of the influence of variable lighting conditions.

This presentation will impact the forensic science community by providing an impartial evaluation of current alternate light sources and chemical detection methods for crime scene investigation. Comparisons of portable forensic methods will be demonstrated whilst highlighting the most befitting method tested for each forensic scenario.

The ability to successfully detect individual biological samples from various evidence substrates is the first tier to an efficacious forensic process. To a great extent, this capability depends on the type and amount of biological specimen present, substrate material, and environmental conditions. Typically, the high variability of forensic circumstances requires access to an extensive amount of materials and equipment to adequately investigate all possible scenarios. Therefore, effective and multifaceted tools capable of detecting biological deposits amidst a vast range of conditions are imperative for a thorough and expeditious crime scene investigation.

In this study, seven different portable alternate light sources ranging from wavelengths of 365nm to 530nm were evaluated. Each light source was evaluated by measuring the ability to detect eight different biological contributions (blood, semen, saliva, urine, 1:1000 diluted blood, 1:3000 diluted blood, dry fingerprints, and oily fingerprints) on nine common porous and nonporous substrates. Each biological contribution was deposited onto each substrate in triplicate. During evaluation, three variable light conditions (no light, low light, and bright light) were used to simulate environmental conditions. In addition, 14 commercially available chemical detection methods were evaluated. Each developing agent was evaluated by measuring the ability to detect biological contributions on various substrates. Each chemical method was tested according to the manufacturer's recommendations, which stated biological specificity and additional developing requirements.

Evaluations for each alternate light source and chemical method were performed 24 hours after the biological contributions were deposited. Three scientists independently evaluated the detection of each biological deposit in triplicate for every substrate and lighting condition using a 1-3-9 rating scale. This measurement scale allows for a clear distinction between performing poorly (1), performing marginally (3), and performing well (9). An aggregate of 216 independent evaluations were made for each type of alternate light source using the 1-3-9 rating scale (1,512 total evaluations). Chemical methods were evaluated on biological contributions depending on the manufacturer's recommendations for specificity. Using the 1-3-9 rating scale, 1,044 total evaluations were performed using chemical developers on nine substrates. In addition to evaluating the detection of each biological deposit, the chemical methods were also evaluated on usability. Usability for this study was defined as "the ease of use in a field setting as a portable technique." Similar to the rating scale for detection, usability was rated as poor (1), marginal (3), or excellent (9).

The alternate light sources evaluated in this study did not significantly detect blood or fingerprints. Contrastingly, the chemical developers evaluated in this study did not significantly detect semen, saliva, or urine. When visualized with 410nm to 445nm alternate light sources, semen, saliva, and urine were highly detectable in most scenarios. Fluorescent fingerprint powder was highly effective at detecting fingerprints and averaged near excellent on the usability rating. Hemascein® was notably effective at detecting all contributions of blood during this study and rated excellent on the usability rating.

This study has shown that relatively few pieces of equipment are required for a forensic unit to effectively detect a large array of biological fluids on many different substrates. Throughout this study, these methodologies have also shown to be portable, resilient, and user friendly.

Forensic Detection, Biological Evidence, Portable Methods

D29 Development of a Rapid Screening Method for the Processing of Sexual Assault Kits

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After attending this presentation, attendees will understand the potential ability to eliminate presumptive testing in favor of a rapid screening method for sexual assault kits, its ability to be achieved after initiating analysis at Y-quantitation.

This presentation will impact the forensic science community by developing a method of rapid screening that will decrease time taken to process a sexual assault kit, which in turn could increase the percentage of reported sexual assaults, which currently stands at 50 percent.

According to the Criminal Victimization Survey of 2010, conducted by the U.S. Department of Justice, approximately 200,000 sexual assault victimizations occur annually with only a 50.0 percent report rate.[1] When a sexual assault is in fact reported, an official statement is taken followed by a thorough forensic examination. This examination is generally performed with the aid of an evidence collection kit, more commonly referred to as a rape kit. The evidence collection kit for a sexual assault generally includes instructions, bags and sheets, swabs, a comb, envelopes, nail pick, blood collection devices, and documentation forms, although the exact contents of a rape kit varies by jurisdiction.[2] These contents are used to collect victim's clothing, possible physical evidence, blood, urine, hair, and other bodily fluids. Due to the vast amount of evidence collected from the victim, it can take anywhere from six to nine months for a laboratory to begin analyzing a single rape kit.[3] The annual backlog of unprocessed rape kits in the United States is estimated to be around 180,000 with a high of 500,000.[3,4,5] The method in which a rape kit is processed is dependent on the laboratory. Typical processing consists of first preliminary testing which includes reagent and microscopic examination. Reagent testing includes, but is not limited to, prostate specific antigen (PSA) test, acid phosphatase (AP) test, human salivary amylase (HSA) test, and a sperm protein (SP) test. The PSA test confirms the presence of semen by the detection of a glycoprotein produced by the prostate gland and this test is found to be more precise than the AP test which detects the presence of the enzyme acid phosphatase which is found in semen.[6] Microscopic examination includes various staining processes of spermatozoa such as the Kernechtrot Picroindigocarmine staining method. Preliminary testing is preceded by the analysis of DNA from samples and swabs. Analysis includes extraction, quantitation, and amplification of DNA followed by the generation of a DNA profile. The objective of this experiment is to develop a method in which sexual assault kits can be rapidly and efficiently screened. This will be studied through the use of different mixture concentrations of male and female DNA to simulate samples currently encountered in sexual assault kits. Samples will undergo the traditional screening method used in crime laboratories including presumptive testing of swabs, staining and the evaluation of slides, etc. The same samples will undergo a Y-quantitation thus eliminating timely presumptive testing. Profile data from the traditional screening method will be compared to profile data for the rapid screening methods chosen. The two methods will be compared with regards to timeliness, efficiency, and cost.

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Rapid Screening, Sexual Assault, Y-STR

D30 Effects of Corrosive Environments on Fractured Surfaces of Stainless Steel Knives

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After attending this presentation, attendees will be informed of the effects real-life situations may have on the fractured surface of a stainless steel knife; for example, the tip of the knife being left behind at the scene, exposed to the elements, left soaking in the victim's blood, and if the hilt of the knife was discarded in a lake or river, or was cleaned with common household cleaning agents.

This presentation will impact the forensic science community by providing effects of different environments and conditions for which evidence can be found on stainless steel knifes, an ongoing project examining several different materials, such as metals, ceramics, polymers, adhesive tapes, and paper.

Thirty-nine Chicago Cutlery stainless steel steak knives were broken using the same load mechanism leaving 39 tips and 39 hilts. Each sample was then weighed and randomized. Using the Analysis, Comparison, Evaluation, and Verification (ACE+V) method of fracture match, each tip was correctly identified and matched to its corresponding hilt. Nine samples were then exposed to blood; three for one week, three for two weeks, and three for three weeks. Nine samples were exposed to a 3.5% solution of saltwater for the same intervals. Three samples were briefly dipped in bleach and three briefly dipped in ethanol and allowed to air dry. Three samples were soaked in a 50% solution of bleach, three in a 50% ethanol solution, three in a concentrated bleach solution, and three in a concentrated ethanol solution, each for two weeks. Three control samples as well as the other half of each of the exposed samples were not exposed. All samples were stored at room temperature during exposure. After exposure, each sample was cleaned, allowed to dry, and reweighed. The ACE+V method of fracture match was used again to attempt to identify the exposed samples to their other half.

Blood samples showed zero sign of degradation and no significant loss of mass after three weeks of exposure. Saltwater samples showed visual signs of oxidation on the side of the sample within just a few hours, but none of the samples showed degradation on the fracture surface. After three weeks of exposure, they experienced an average loss of mass of 0.25%. All blood and saltwater samples were correctly identified using the ACE+V method of fracture match. None of the ethanol samples showed any visual sign of degradation nor did they experience any change in mass. One sample, however, was rendered an inconclusive result during matching using the ACE+V method. The three dip-and-dry bleach samples showed neither degradation nor any change in mass. The samples in the 50% solution of bleach showed a 3.26% increase in mass due to oxidation while the samples in the concentrated solution experienced an 8.47% increase in mass. One of the samples in the concentrated solution was yielded an inconclusive result using the ACE+V method. All other samples were correctly identified.

In order to address the recommendation expressed by the National Academy of Sciences, this project sought to provide preliminary

investigation on the impact of different environments and conditions for which evidence can be found. This is an ongoing project examining several different materials including, but not limited to, additional metals, ceramics, polymers, adhesive tapes, and paper.

Work at the Ames Laboratory was supported by the U.S. Department of Energy Science Undergraduate Laboratory Internship (SULI) Program under its contract with Iowa State University, Contract No. DE-AC02-07CH11358. This research project was also partially funded by the U.S. Department of Justice, National Institute of Justice at Ames Laboratory, under Interagency Agreement number 2011-DN-R-0230.

Fracture, Corrosion, Steel

D31 Poppy Tea: An Update of its Abuse in the United States

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After attending this presentation, attendees will gain a greater awareness of the growing trend of poppy tea abuse in the United States and an understanding of the potential morphine content found in poppy tea.

This presentation will impact the forensic science community by increasing awareness of the growing trend of poppy tea abuse.

Poppy tea, an infusion made from the seeds or (more commonly) the un-lanced, dried pods of the opium poppy, is hardly a novel product. It has been used ceremonially and medicinally in Eastern and Western cultures for centuries. However, as opiates such as morphine and heroin increased in popularity both as medical remedies and drugs of abuse, poppy tea faded into relative obscurity. Thanks to information available on the internet, abuse of poppy tea has rebounded in recent years. Simple recipes for its preparation are easily found on various websites. Dried poppy pods and seeds are also readily available from Internet vendors worldwide, including within the United States.

There are several varieties of *Papaver somniferum*, the opium poppy, that are cultivated for both licit and illicit purposes. The physical characteristics and alkaloid profiles of these poppies vary by growing region. In Mexico and South America, the opium poppy pods are small and rounded, and generally less than three centimeters in diameter. The poppy pods of the Southwest Asian region (Afghanistan, Turkey) are more elongated, with diameters of around two centimeters. Southeast Asian poppy pods, found in countries such as Thailand and Myanmar, are typically larger than the varieties found in the Americas or the Southwest Asian countries, with diameters of about four centimeters. In addition to these size differences, each region's poppy pods also have varying alkaloid contents.

Users of poppy tea identify the varieties of poppy pods by their flowers, seed color, and pod size and shape. Popular tea varieties include Hens and Chicks, Danish Flag, Persian White, and Giganteum. The Giganteum (or Giganthemum) variety has extremely large sized pods ranging from two to nearly six centimeters in diameter. Many Internet vendors refer to these pods as "jumbo" or "mammoth" sized. Some of the U.S.-based sites also boast of their domestically grown "jumbo" pods. One website claims to carry "Arizona's Best" poppy pods.

In the last few years, several fatal and nonfatal morphine overdoses related to poppy tea have been reported in the United States, Canada, and the United Kingdom. Canada has seen a growing problem with "doda" (or "dode"), which is a term for the powder made from ground opium poppy pods and seeds. Recently, poppy pods have been smuggled into Canada from the United States. These developments prompted an investigation into the possible alkaloid content in poppy tea. Unusually high morphine contents were detected in batches of tea prepared from a popular variety of poppy pod illicitly grown in the United States. The teas also contained varying levels of other opium alkaloids including codeine. Many users in the United States incorrectly assume that the use of poppy tea is both legal and a safer alternative to heroin. However, given the variety and levels of opium alkaloids found in poppy tea, the potential for abuse, addiction, and overdose is significant.

Forensic Science, Poppy Tea, Opiate Abuse

D32 A Case Study of the Murder Weapon Identification Through the Trace Evidence From Crime Scene Investigation

Choi Yong-Bok, PhD, Chungnam Provincial Police Agency, 301-708, Daejeon, KOREA*

After attending this presentation, attendees will learn important aspects of collecting trace evidence with other circumstantial evidence at the same time.

This presentation will impact the forensic science community by showing the importance of collecting and analyzing trace evidence to quickly determine the cause of death.

Today, many investigators only think that an individual identification is possible through DNA analysis from body secretions such as saliva and sperm, and hair and epithelial cells which are found in a crime scene; they just try to search this to find criminal(s) from the fingerprints on the evidence. If they can't find this evidence, they would focus on searching trace evidence which is used less during investigations.

Trace evidence examination has come from Edmond Locard's (1877~1966) observation in France, 1910; "with contact between two items, there will be an exchange." Therefore, while investigating criminal cases, trace evidence has to be used like other evidence such as fingerprints.

On Oct. 2009, a victim (A, 28-year-old, female) is murdered in the master bedroom and a suspect (B, 41-year-old, male) who killed himself in the bathroom next to the master bedroom the city of Bo-Reong, Chung-nam province. While searching the crime scene, the cause of death of the victim was quickly judged as cervical compression asphyxia. Trace evidence was collected from the victim's neck, the cord which was concluded to have been used during the criminal activity, and the suspect and victim's hands, to make clear what tools the suspect used during the crime. After a postmortem examination, the cause of death is determined as cervical compression asphyxia. While there is a scratch in the epithelial cells on a horizontal line, there was not a specific injury in the cervical muscle. Therefore, it is possible the victim was strangled by ligature, which squeezes the throat using a cord; but it is also possible to squeeze the victim's throat using a hand while at the same time the suspect was squeezing the victim's throat using a cord, as there are a few scratches of tiny epithelial cells on the neck.

In this case study, fabric which was collected from the victim's neck was compared with fabric (trace evidence) collected from the cord used during criminal activity and analyzed to see if they were the same. Analysis found: (1) the fabric of the cord and the fabric collected from suspect's hand and neck were same; (2) the blue fabric of the cord and the blue fabric on victim's neck were the same; (3) the red fabric of the cord and the red fabric on victim's neck were the same; and, (4) the bleaching red fabric to collect the tape on victim's neck and the bleaching red fabric of the tape on the cord were the same. These are the scientific proofs that the crime was committed using the cord.

Today, trace evidence is less used in crime investigation because it is not interesting. In many cases of murder and unnatural death, trace evidence has not been collected and investigators depend on a result of autopsy to determine the cause of death. However, like the example case, the scientific proofs using trace evidence is as important as postmortem examination.

As many crime cases are investigated, the trace evidence has to be collected and it is possible to provide individual identification with a high degree of certainty. If the trace evidence is used with other fragmentary evidence during the investigation, this reasonable method to investigate and solve a crime will be used frequently.

Trace Evidence, Fabric, Autopsy

D33 Non-Destructive DNA Collection From Handled Documents Using an Electrostatic Detection Device

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The goal of this presentation is to determine if general use of an Electrostatic Detection Device (EDD) on handled documents could be used to collect touch DNA from paper documents in a non-destructive manner.

This presentation will impact the forensic science community by showing the potential of an electrostatic detection device in the lifting of touch DNA from handled paper documents with a statistically significant difference between substrate types.

Evidence collected for forensic testing can be defaced or even destroyed by the collection processes, reducing the integrity of the evidence and creating artifacts that could impact downstream analyses. An example of destructive testing is the collection of touch DNA from handled paper documents using a standard swabbing method. The purpose of this study was to determine if the general use of an EDD on handled documents could be used as a means to collect touch DNA from paper documents in a non-destructive manner. An EDD apparatus uses a charged Polyethylene Terephthalate (PET) sheet and is commonly used by document examiners to develop imprinted writing and fingerprints on paper documents without the direct treatment of the document itself. The question was raised if DNA deposited onto paper documents could be lifted by the PET sheet of the EDD apparatus.

Fingerprints from three donors were deposited onto five different paper document substrates: copy paper, newsprint, cotton (resume) paper, magazine print, and currency. Fingerprints were deposited onto each type of substrate on separate days in order to regenerate similar touch conditions. Each document substrate was subjected to processing for fingerprints using an EDD. As a control, an additional fingerprint collection of each substrate was cut from each document. The cut samples were subjected to the same conditions and processing as the test samples. Post collection, the substrates were visually inspected for damage. Cutting of substrates resulted in visible substrate defacing; however, the substrates collected by the EDD did not have any visible defects. Post processing, the PET sheet of the EDD was inverted and samples were collected from locations where fingerprints were visualized using a moistened cotton swab.

All samples were extracted (Silica Particle), quantified, concentrated, amplified for Short Tandem Repeats (STRs), and analyzed in accordance with approved laboratory procedures. All samples quantified below the detectable threshold of the system. Therefore, sample extracts were concentrated from 50uL to 5uL and amplified using the highest quantity of template useable by the STR amplification kit. The end result was the identification of 11 full profiles and 27 (out of 75) useable profiles (above 62.5% of profile resolved).

In addition to the ability to generate useable profiles, this collection technique presented two interesting variables: there was correlation between percent profile detected and donor, and also a correlation between percent profile detected and paper substrate types. Variation between donors was expected due to the difference between individuals and their ability to shed DNA. Between substrates, there was a statistically significant difference between all five substrates. Cotton paper returned the highest quantity of profile averaging of 81% across all donors, with other substrates significantly lower: copy paper: 48%; magazine print: 32%; newsprint: 45%; and, currency: 36%. In comparison to cut samples, the EDD device collection was similar and in the instance of cotton paper, more effective than cut sampling.

This study has shown the potential of an EDD device in the lifting of touch DNA from handled paper documents with a statistically significant difference between substrate types. This technique also leaves no visible defacing on the document and does not interfere with downstream questioned document analyses, greatly impacting the forensic community at large

Touch DNA, Questioned Documents, Evidence Collection

D34 Object Orientation to Minimize Metallic Artifacts in Multi-Detector Computed Tomography and Maximize Resolution With Plain Radiography

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After attending this presentation, attendees will have a better understanding of basic imaging principles that can be used to improve image quality in forensics that are not as easily applicable in a clinical setting.

This presentation will impact the forensic science community by providing two imaging procedures that will improve image quality. Specifically, the first, related to Multi-Detector Computed Tomography (MDCT), will minimize streak artifact most notable when the data is used to create facial reconstructions. The second, relative to plain radiography, provides a method to avoid missing an occult fracture in a newborn or infant.

Introduction: Patient positioning and radiation dose limitations associated with clinical radiology are not a consideration in forensic imaging. Eliminating these restrictions provides the opportunity to utilize basic imaging principles to improve image quality that are not as easily applicable in the clinical setting. Two examples relative to object orientation will be discussed in the following presentation. The first, regarding MDCT, considers streak artifact created by dental fillings and most notable on three-dimensional reconstruction. When performing a skull examination on a live patient, there are few positioning options. The resulting 3D skull reconstructions of a patient with numerous bilateral premolars and molar fillings will exhibit streak artifacts projecting out of the mouth at right angles to the teeth. The second example relates to the X-ray tube focal spot in general or plain radiography. An object smaller than the focal spot size will appear as a blurred structure on the resulting image. Therefore, the dimensions and orientation of the rectangular focal spot within the X-ray tube is important when positioning an object to maximize resolution of subtle defects such as an occult fracture in a premature or newborn infant. However, in the clinical setting, due to monitoring and life support lines that might be attached to the patient, there may be limited choices for X-ray tube orientation. In addition, concern over the radiation dose will limit the technical factors and image receptor selection.

Materials & Methods: For the MDCT component of the study, the skull of an individual who died in 1886 and had numerous gold fillings was used to demonstrate orientation in an MDCT examination intended for a 3D reconstruction. The detached skull provided numerous positioning options. The skull and attached mandible were positioned with the sagittal suture parallel and perpendicular to the x-ray/detector rotation. In order to demonstrate the importance of positioning relative to the shape of the X-ray tube focal spot, a preserved fetal pig with an induced transverse fracture of the left third rib was radiographed. The specimen, with a crown-rump measurement of 29cm, was positioned with the fracture oriented both perpendicular and parallel to the smaller dimension of the focal spot. In addition, 40 kilovoltage-peak, kVp, much lower than would be clinically acceptable, was used for all the exposures.

Results: In the MDCT study, when the sagittal suture was aligned parallel to the tube/detector rotation, the streak artifacts were projected posterior to the teeth and not visible external to the mandible and maxillae. With the plain radiography, the occult rib fracture was more clearly demonstrated when the fracture was oriented parallel to the smaller dimension of the X-ray tube focal spot.

Conclusion: With fewer limitations regarding patient positioning and radiation dose, basic knowledge of imaging principle can be employed in forensics to improve image quality. Object orientation in MDCT can reduce streak artifact, improving 3D data sets used in facial reconstruction. In plain radiography, low kVp and object orientation relative to the shape of the focal spot improves image quality.

MDCT, Facial Reconstruction, Radiography

D35 Tampon Pain and Injury From the Perspective of the Adolescent and Adult User

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After attending this presentation, attendees will understand the potential for pain and injury caused by a tampon inserted by the female user during menses. Furthermore, tampon pain and injury are compared in those using tampons 100% of their menses from those using tampons along with pads.

This presentation will impact the forensic science by providing results of a survey of 125 female subjects using tampons. It is significant to the forensic community because tampons are implicated as a rival hypothesis for injury to the female genitalia in sexual assault trials. Results of this study provide further scientific evidence in which to understand the question of tampon injury both in those using tampons 100% of the time and comparing those 100% tampon users to those using tampons part of the time in addition to using pads.

The presence and character of potential tampon injury to the genitalia has been a nagging question beginning in the 1930s when tampon use became more common. Some have said tampons do not result in hymenal clefts even in virgins.[1] Others have demonstrated that hymenal clefts occur in even the sexually inactive and the possibility of those clefts being tampon induced cannot be excluded.[2] Tampons without applicators were considered in the etiology of vaginal lesions by others.[3] Recently, it has been purported, without research support, that "injury to the posterior fourchette during tampon insertion is not uncommon;"[4] however, genital injury from tampons is not clearly or consistently evident in the research literature.[1,5,6] Also, the literature present is primarily from the perspective of the provider. Tampon-induced pain or injury from the perspective of the adolescent and adult tampon user contributes clarity to this growing literature on potential tampon-induced injury.

In this study, 125 sexually active adolescent and adult females were chosen by snowball convenience selection in the United States during 2011–2012. The survey was developed by experienced sexual assault examiners. The format of the paper-and-pencil questions required simply a yes or no and explanation as participants answered questions about their tampon or pad use. Postmenopausal women also answered from recall about the years of their tampon use. The results were tabulated into spreadsheet and will be further analyzed with descriptive results and inferential testing of the difference between the two groups – those using tampons 100% of the time and those using tampons and pads. Those using pads only served as a control group.

Three groups were surveyed: those using pads only, those using pads and tampons and those who used tampons only. Results will reveal the tampon type—straight or umbrella, plastic or board applicator and usage in the two groups using tampons. Also demonstrated will be the presence of pain or injury on tampon insertion, tampon wearing, and tampon removal, or if the tampon user was ever told by the provider during an exam that she had an injury to her genitalia. If pain was present, the user described the cause of the pain and the treatment she found which gave relief.

In conclusion, this study provides evidence from the perspective of the tampon user regarding pain, potential injury, and how it was resolved. Furthermore, results reveal whether those using tampons 100% of the time are any different in their pain and injury from those using tampons part of the time, in addition to pads. Data from this study contributes to the growing research evidence regarding tampons and genital injury.

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Tampon, Genital Injury, Postmenarchal

D36 Characterization of Legal Highs and Their Pyrolysis Products

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After attending this presentation, attendees will become familiar with methiopropamine (3-MPA) and 6-(2-aminopropyl)benzofuran (6-APB), two of the latest legal highs that are being sold legally, both online and in head shops across the country. The contents of these drugs have been verified; therefore, this presentation will focus on the by-products formed when MPA and 6-APB are smoked. Attendees will learn about the process of pyrolysis and the importance of identifying the pyrolysis products of legal highs.

This presentation will impact the forensic science community by informing attendees how law enforcement and health professionals are grappling with the hazards presented by the current proliferation of legal highs. Never intended as pharmaceuticals, the pharmacology of these drugs are unknown. Therefore, treatment for patients who have taken these drugs is limited to symptomatic care. This project focuses on the next level of harm, the pyrolysis products formed when the legal highs are consumed by insufflation. This information can be used not only to aid in identifying drugs in a laboratory setting and help them to become scheduled, but may also help to save lives.

Legal highs are any mind-altering substance produced and sold in a manner to avoid the legal consequences of illicit substances. They have been advertised and sold as Spice, Bath Salts, plant food, and research chemicals. They are often analogs or derivatives of classical drugs of abuse such as cathinones and amphetamines, but do not fall under the Federal Analog Act as they are marketed "not for human consumption."

The harmful substances are not limited to the primary components within the drugs, but can include potentially toxic by-products that result when the drugs are smoked. Heroin, cocaine, and methamphetamine are all known to produce toxic by-products as a result of pyrolysis.

As part of an ongoing research project, legal highs have been purchased from local head shop's Internet web sites. Some of the substances identified include methiopropamine, 6-(2-aminopropyl)benzofuran (6-APB), 5-(2-aminopropyl)benzofuran (5-APB), and UR-144 (1-Pentylindol-3-yl)-(2,2,3,3-tetramethylcyclopropyl)methanone). The objective of this project is to identify the pyrolysis products from these legal highs which may play a significant role in the pharmacological and toxicological effects of these drugs.

The powder is loaded onto an aluminum foil boat and placed in a 25ml Erlenmeyer flask. The bottom of the flask is heated with a disposable cigarette lighter until the sample is black and no more fumes are visible. The aluminum boat is removed and the residues remaining in the flask are dissolved in methylene chloride. The residues are analyzed by gas chromatography and mass spectroscopy.

The pyrolysis products of methiopropamine and 6-and 5-APB have both been analyzed. Four pyrolysis products were presumptively identified from the pyrolysis of methiopropamine. Those products include the methylation and demethylation of the nitrogen atom. Both of these products are consistent with the pyrolysis of methamphetamine, an analog of methiopropamine. Ethylation of the nitrogen atom also occurred, which may indicate the use of ethanol during the synthesis process.

The pyrolysis of APB was more complex, producing nine pyrolysis products. Of those, six have been tentatively identified. Four of those products are consistent with the pyrolysis of methamphetamine: oxidation of the carbon chain and formation of a double bond followed by removal of the nitrogen group. The other two products are consistent with the pyrolysis of the benzofuran ring: addition of a methyl group to the ring followed by a combination reaction of two APB molecules.

Methiopropamine (MPA), 6-APB, Pyrolysis

D37 The Application of Laser Scanning for Visualization Within a Courtroom Environment

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After attending this presentation, attendees will gain a better understanding of how laser scanning and three-dimensional (3D) modeling can be used for visualizing forensic evidence within a court of law.

This presentation will impact the forensic science community by determining the advantage laser-scanned evidence has compared to traditional photographic methods for reconstructing a sequence of events within a court of law.

In homicide cases, skeletal trauma can provide evidence for the specific conditions of individuals' death. Forensic anthropologists can use skeletal trauma to reconstruct the final sequence of events for an individual. Photography is then utilized as a way of interpreting this evidence within a court of law.

Photography and digital imaging are the standards for documenting forensic evidence. The use of images is important in the investigation process and allows reconstruction after the scene has altered. More recently, technology from interdisciplinary research is being adapted to record evidence at a crime scene. A laser-scanned scene can be transformed to create a three-dimensional (3D) walkthrough. Thus, a 3D model can be taken into a courtroom as an alternative to photographs. This concept of visualization can also be used to replicate forensic evidence, especially that which is too sensitive to take into a court of law. Laser scanning also allows an enhanced visual walkthrough of events that can be viewed live in front of a jury.

This research compared photographic methods against laser-scanned data. First, several human archaeological skulls were recorded that displayed signs of traumatic damage. These skulls replicate similar blunt and sharp force traumatic damage seen in present populations. In an actual forensic investigation, this type of evidence is highly sensitive for the parties involved. Photography and laser scanning allows sensitive images to be displayed without bringing the actual evidence into the courtroom.

The present study recorded the human archaeological skulls using photographic and laser scanning techniques. The non-contact 3D scanners record surfaces by using basic triangulation. The scanner records an object by building a series of point clouds as a laser line passes over it. This research utilized the a laser scan arm and a compact 3D scanner for comparison against photography. The laser scan arm has an accuracy of up to 50 micrometers (50µm) (0.002") with a repeatability standard deviation of ± 0.002". The laser line has a scan width between 34mm and 60mm and a depth of field of 85mm. The compact 3D scanner has an accuracy of 0.1mm with a resolution of 968 x 644 8 bits and an 18-55mm lens. For photography, a digital SLR camera with an 18-70mm lens was utilized.

The research showed that laser scanning is an extremely useful tool for future applications when replicating sensitive evidence. Laser-scanned data allows information to be captured more accurately than photography. Furthermore, within the editing processes, results show that actual evidence is not being removed. This research also shows the 3D model allows for more accurate visualization than photography. This novel technique has the ability to be manipulated live by rotating and zooming into specific areas of interest. Laser scanning also relays technical information within a court to non-technically-minded people.

Laser Scanning, Visualization, Courtroom

D38 The Practice of Torture Known as Falaka: Evaluation of Physical and Psychological Outcomes in Asylum Seekers

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After attending this presentation, attendees will learn some peculiarities of the torture method known as "falaka," still extremely popular throughout the Middle East and some African countries.

This presentation will impact the forensic science community by giving insight on the importance of a thorough medical examination, including medical-forensic support on asylum seekers and refugees, to certify the outcomes of this method of torture.

Introduction: The goal of this presentation is to analyze a torture method known as "falaka." This method is extremely popular and has existed since the Ottoman Empire to recent times, throughout the East and Middle East. Because this is a very common practice, depending on the geographical area, it is sometimes called: *falanga, falaqa, fallagas, bastinado*. Falaka is the most common term which identifies the repeated application of blunt trauma to the feet (more rarely in the hands or hips), usually practiced with a truncheon or a similar weapon. The most severe complication of falaka is the compartment syndrome, which can cause muscle necrosis, vascular obstruction, or gangrene of the distal portion of the feet or toes. Because lesions are often limited to the soft areas (fractures are rare), CT and MRI are the best methods to document them, but a medical examination in the acute phase can be crucial.

The SaMiFo (Health for Forced Migrants) is a health service designed for asylum seekers and refugees, created in collaboration with the Local Health Unit (LHU) of Roma A., in operation since 2007. The SaMiFo aims to facilitate and monitor all health and social services provided by the LHU. The medicolegal examination which certifies the outcomes of torture is the last step of a diagnostic clinical course (Unit VdT-Victims of Torture) that the patient begins at SaMiFo.

Materials and Methods: The data of our research are related to the period from January 2011 to June 2011, during which the SaMiFo followed 1,500 applications for international protection. Seven percent of applicants had physical and psychological signs of torture and they were therefore referred for medicolegal examination.

Review of 100 cases was completed to include an introductory interview, the analyzing of all the clinical examinations to detect previous diseases, the threatening of the health of the applicant in case of

repatriation, and then the examination to evaluate any injuries. The way in which the clinical examination is carried out and the search for physical evidence are indicated in the Istanbul Protocol.

The presence of a post-traumatic stress disorder is very frequent in this group and it is diagnosed by the Symptom Checklist 90 – Revised, and the Harvard Trauma Questionnaire 67 (HTQ). The HTQ consists of a guided interview that begins by evaluating 46 traumatic experiences. The second section of the interview includes two open-ended questions on experiences perceived by the refugees as by far the worst ever experienced. The third section investigates the experience of trauma or abuse, which are capable of causing brain damage. The fourth section then explores 40 symptoms related to trauma and torture (16 of which related to the diagnostic criteria of DSM IV). As it is a transcultural questionnaire, it has become a widely used tool and reference point for many research fields.

Discussion and Conclusion: Considering the local migration, Africa is the country most represented in this research. Women represent 14% of the sample. Almost all asylum seekers have been beaten, 17% underwent falaka, all women have suffered sexual violence. Twenty-four percent have a chronic post-traumatic stress disorder and all patients report anxiety and adjustment disorders. Different methods of torture are used simultaneously; in fact, in falaka cases studied 40% were victims of sexual violence, 50% had signs of cigarette burns on the body, and more than 60% underwent violence in prison.

The percentage of cases of falaka of our study is consistent with data from international literature; however, there is a significant difference in the countries of origin. Africa is the most represented country in this study; other global regions where this kind of torture is practiced are the Middle East, particularly Iran, Iraq, Syria, Turkey, and the countries of the Indian region, especially Bangladesh.

Another important point concerns the long-term outcomes. In the past, it was believed that this method did not leave signs of torture. It has now been shown that a physical examination performed by medical experts and the use of imaging techniques may identify lesions that, with a high level of probability, can be considered a direct consequence of exposure to this practice.

Falaka, Methods of Torture, Asylum Keepers

D39 Differentiation of Human Subjects Based on Scent Profile

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WITHDRAWN

D40 DNA Backlog Reduction by Triage

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The goal of this presentation is to provide attendees with information regarding a different path for reducing DNA backlog within their respective laboratories, one which has immediate, profound impact, and relatively little cost. After attending this presentation, attendees will have appreciation for a new approach to solving this growing problem in the forensic community.

This presentation will impact the forensic science community by describing the problem set in dealing with DNA backlogs as a result of the significant number of sexual assault cases. This is further complicated by Congressional intervention mandating the reduction of sexual assault cases pending forensic analysis. Several potential tactics will be discussed that could be employed to reduce DNA backlog, as well as describing how the employment of these methods resulted in significant policy changes which benefitted both defense and prosecution by reducing turnaround times.

When most prosecutors, police administrators, and laboratory personnel think of the DNA Reduction Act and the money spent to reduce DNA backlogs, they immediately think of increasing throughput at the laboratory end. This means an increase in manpower and equipment resources that plague governmental institutions in seeking additional revenues. From 2005 – 2009, the year-end backlog of cases at publically funded forensic crime laboratories rose over 200%. While the sheer numbers of submissions increased, so did the number of completed cases. According to National Institute of Justice (NIJ) statistics, crime laboratories are just not able to keep up with the flood of ever-increasing DNA submissions.[1] The federal funding made available through the DNA Initiative has helped state and local governments increase the capacity of their DNA laboratories to decrease backlogs. Without the funds to purchase automated instrumentation, hire new personnel, and validate procedures that are more efficient, the backlog problem would be much worse. Capacity at the laboratories has not yet come close to the demand for DNA testing. Until the demand is met, there will continue to be backlogs.

By published statistics, about $75 million is granted by the Federal Government annually to local and state crime labs in support of the DNA backlog reduction program. This figure is on top of the budgeted costs for initial resourcing and staffing of the supported laboratories. A DNA sample collected and analyzed by a laboratory in support of criminal justice work costs between $800 – $2,500 per sample, a cost per additional suspect identified is $4,502, and a cost per additional arrest is $14,169.[2] Many government laboratories have taken the step to contract with commercial DNA labs to reduce these backlogs adding to the financial burden. Until the demand is met, there will continue to be backlogs. The presentation will cover the necessary statistics regarding the cost of DNA backlogs, and DNA testing as a basis for the cost savings associated with this tact.

This presentation will discuss the methods and rationale used at a major U.S. crime laboratory where, by using a panel of experts, we were able to quickly and efficiently reduce the physical DNA backlog by up to 40%. In addition, we will be discussing the metrics and rationale used in expeditionary laboratory environments to ensure that on the highest payoff cases, the most probative evidence is front-loaded, yielding real, actionable information for investigations.

The presentation will also address additional outcomes of the triage efforts, as well as some amusing, and less-than-probative, laboratory requests that were collaterally eliminated as a result of this effort.

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2. Roman, A., Reid, S., et all (2008) The DNA Field Experiment: Cost-Effectiveness Analysis of the Use of DNA in the investigation of High-Volume Crimes: U.S. Department of Justice research report.

DNA, Reduction, Triage

D41 Comparison of DNA Yield From Different Soft Tissues of Decomposed Human Body at 4°C and -80°C

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After attending this presentation, attendees will understand the importance of proper selection of soft tissue from decomposed bodies and proper conditions for preserving it for DNA analysis, especially in developing countries like India.

This presentation will impact the forensic science community by giving guidance about selecting the proper tissue for DNA analysis in case of decomposed bodies and the suitable preservation condition for such tissue. It can improve the outcome of conventional methods of DNA analysis in case of decomposed bodies and can give better results while identifying unknown decomposed bodies by DNA analysis.

It is always difficult to identify dead bodies by just external features in case they are decomposed. Identification by DNA analysis becomes the most important method of identification in such cases. DNA analysis can be easily performed on fresh tissue samples because of very little, if any, chances of degradation of genomic DNA, but it is of real concern to decide the best tissue for DNA extraction and analysis, and then to preserve it at the right conditions for analysis in case the body is decomposed.

Conditions required for preservation of tissues from dead bodies for DNA analysis is of great importance especially in developing countries like India where proper facilities for preservation of dead bodies or tissues are not available at primary and secondary health care centers and even at some of the tertiary health care centers. Also, preservation conditions become important in tropical countries with warm weather which hastens the process of decomposition. Stored samples encounter problems of degradation of High Molecular Weight (HMW) genomic DNA depending upon degree and nature of storage.

The present study is focusing on determining the best suitable soft tissue (among brain, kidney, heart, and muscle) for DNA analysis in case of decomposed dead bodies and the better temperature (among 4°C and -80°C) for the preservation of soft tissue for DNA analysis. The study was conducted on 16 different decomposed dead bodies from which four tissues (brain, kidney, heart, and muscle) were selected for sample collection. The collected samples were preserved at two different temperatures (4°C and -80°C) without any preservative for one month after which DNA was extracted using Phenol Chloroform extraction method (organic method) and the DNA yield was calculated using Spectrophotometer. Quality of extracted DNA was checked using gel electrophoresis, PAGE, and PCR.

The yield of DNA was more at -80°C than at 4°C for all the four tissues collected from 16 bodies. While analyzing the DNA yield for individual tissues, it was found that the yield was remarkably higher in brain tissue followed by heart, then kidney and least for muscles in all the cases at 4°C as well as -80°C. The DNA extracted from all the tissues at both the temperatures was found to be of good quality for amplification purpose with brain having the highest amount of HMW DNA.

This study suggested that deep freezing at -80°C should be preferred for preserving tissues for DNA extraction wherever available and brain must be the preferred soft tissue followed by heart, kidney and then muscle for DNA analysis at both normal freezing (4°C) and deep freezing (-80°C) temperatures.

DNA, Decomposition, Dead body

D42 Recovery Of Human DNA Profiles From Poached Deer Remains

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After attending this presentation, attendees will have a new understanding of how wildlife crime can be investigated more efficiently with improved conviction results. The described method will also be applicable to other types of wildlife crime.

This presentation will impact the forensic science community by allowing wildlife crimes to be investigated and prosecuted, with the use of human DNA giving the described test wider usage and acceptance in court.

The role of forensic science in non-human crime, mainly wildlife crime, is underused. Crimes against animals have been demonstrated to be linked with other organized crime due to the high value of the crime combined with the low levels of prosecution. It is therefore important that new methods be developed that will maximize the recovery of evidence from wildlife crime as well as the probative value of that evidence for criminal investigations.

Poaching is a worldwide crime that can be difficult to investigate due to the nature of the evidence. Previous studies have focused on the identification of endangered species in cases of poaching. Difficulties arise if the poached animal is not endangered. In the United Kingdom (UK), deer have hunting seasons whereby they can legally be hunted. Therefore, identification of deer alone has little probative value as samples could have originated from legal hunting activities in season. After a deer is hunted, it is common practice to remove the innards, head, and lower limbs. The limbs are removed through manual force and represent a potential source of human "touch DNA."

The potential to recover and profile human autosomal DNA from poached deer remains was investigated. Samples from the legs of 20 culled deer were obtained using DNA minitapes. DNA from samples was extracted, quantified, and amplified to determine if it would be possible to recover human STR profiles.

Initial profiling required the use of Low Template (LT) analysis. This method is not accepted worldwide and a revised protocol was developed that did not require the use of LT analysis. The revised protocol involves combining the DNA extracts from all legs (or samples) relating to the same simulated poaching incident followed by concentration of the DNA sample. This allows the maximum amount of recovered DNA to be added to the human STR multiplex.

Near complete profiles that provided a high level of discrimination were obtained. The majority of the samples provided at least a partial profile. Incidents of drop-in were minimal considering the isolated location of the sample recovery.

This project demonstrates the recovery of human touch DNA from poached animal remains in a simulated study. This test is far superior to those targeting animal DNA due to the recognized validation of human STR testing as well as the potential for the results to be uploaded and searched against a human DNA database, for example, the UK National DNA Database. There is the potential for this test to be used in relation to other species of poached remains or other types of wildlife crimes. This is the first time that human STR profiling has been successfully applied to touch DNA in regard to wildlife crime.

Poaching, Wildlife Crime, Human STRs

D43 The Use of Finite Element Head Models to Predict Skull Base Fracture

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After attending this presentation, attendees will be presented with the finite element method, its principles and its applications in forensic sciences focusing on a skull base fracture case, showing the possible interest in some forensic cases of the use of this method.

This presentation will impact the forensic science community by understanding the use of finite element models, their possible application in practice, and also, through the particular case reported, to be careful when a body is found floating in a river without external evidence of injury, not to conclude too fast on the cause of death.

It can be a fundamental problem in forensic investigations to establish whether a head injury is the consequence of an accident or an assault. Close examination of the wounds may help to understand the mechanism of injury, but sometimes doubt remains and it may be particularly difficult to differentiate the consequence of a fall from the consequence of a blow. During the second half of the 20th-century, engineers have tried to develop mathematical models using fundamental Newtonian principles and experimental observations to predict the mechanisms of head injury for a given scenario. Yet, inaccuracies can arise from these models in which tolerance to impact originates from various sources, including experiments on animals,

human cadavers, anthropomorphic dummies, and human volunteers. A proposed alternative method for assessing the consequence of a given head impact scenario is the use of finite-element models of the human head. Computer models are increasingly proving to be an alternative to complicated, practically unfeasible, or unethical (human or animal) experiments. The finite-element method, which is a mathematical method for solving complex physical problems on domains with complicated geometries, is commonly used in constructing such computer models. The finite-element modeling technique offers the advantage of being able to model structures with intricate shapes and indirectly quantify their complex mechanical behavior at any theoretical point. Because the finite-element method uses the theories of elasticity and static equilibrium, the effects of multiple external forces acting on a system can be assessed as physical events in terms of deformations, stresses, or strains. A finite element model of the human head has been developed in Strasbourg University to predict skull fractures and brain injuries. The geometry of the inner and outer surfaces of the skull was digitized from a human adult male skull. The main anatomical features modeled were the skull, falx, tentorium, subarachnoid space, scalp, cerebrum, cerebellum, and brainstem. For the CSF, a Lagrangian formulation was selected and the brain–skull interface was modeled by an elastic material validated against the *in-vivo* vibration analysis. The scalp was modeled by a layer of brick elements and surrounds the skull and facial bone. Globally, the model consists of over 13,000 elements. Its total mass is 4.7kg. Tolerance limits were identified relative to DAI, subdural hematoma, and skull fracture with a risk of occurrence of 50%. The complex geometry of the skull, including the evolution of the skull thickness throughout the skull, and the reinforced beams which play an important role in its dynamical response to impact have been taken into account. Previous published papers have dealt with the use of this finite element head model in forensic sciences for falls and gun injuries. This paper illustrates its possible use for predicting skull base fractures through the report of the case of a woman who was found floating in a river near Strasbourg, next to the boat where she used to live. External examination showed no particular injury. Autopsy showed a right low parietal head impact and a transverse fracture of the skull base with traumatic dilacerations of the two internal carotid arteries. Death was due to "drowning" by the victim's own blood which had obliterated the trachea passing through the skull base fracture into the pharynx. A finite element method reconstruction of the fall and the head impact on the dock where the ship had stopped was made, showing how the victim could have fallen to create her skull base fracture, thus confirming the possibility of an accidental death.

Finite Element Model, Skull Fracture, Drowning

D44 Investigation of Real-World Blunt Head Trauma Using a Commercial Skull/ Brain Surrogate

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The goal of this presentation is to educate the forensic community on a potential surrogate available for assessment of blunt force trauma, and how to critically evaluate such surrogates.

This presentation will impact the forensic science community by displaying data, laboratory procedural background, and morphological results related to human head blunt-force impacts that will provide insight into the applicability of blunt-force trauma surrogates as tools for human skull fracture pattern prediction.

Often, the circumstances leading to skull trauma as a result of blunt impacts may be unknown due to the lack of witnesses, the inability of the injured to recall the events leading to the trauma, or even the death of the injured party. In one real-life incident, a worker investigating the outlet area of a hydraulic pump that was removing fluid from a well sustained serious head and brain injuries as fluid pressure reached the critical value needed to blow the cam-lock cover off of the pump outlet, which then struck him in the head. For such cases, human head surrogates have been developed that allow laboratories to simulate such scenarios. Thali et al. (2002) introduced a novel headform, commonly known as the "skin-skull-brain model," which was developed with the intent of observing the subjective morphological results of blunt head traumas.[1] The focus of this study was to attempt to recreate the aforementioned real-life injury on a similar surrogate. In doing so, the goal was to attain a better understanding of the circumstances surrounding the injury by comparing experimental morphological results to injury evidence, and perhaps to gain insight into the validity of the surrogate for similar blunt trauma events.

For impact testing, the human head was represented using a surrogate similar to the Thali model, consisting of a polyurethane sphere to simulate the human skull (SYNBONE) and 20% ordnance gelatin as the brain simulant. The surrogate was hung in a thin net to simulate head movement as a reaction to impact force. In an attempt to simulate the head injury event, a 1.5 lb. cam-lock cover which matched the real-life blunt trauma projectile was launched from a 2.75" section of pipe on the end of an air cannon to produce a direct impact to the surrogate. Based on the available information pertaining to the impact event, three possible impact conditions were tested: the cam-lock cover was fired from 25" at 94.8 psi (gage) while loosely fitted to the cannon pipe, from 15.25" at 95 psi with the cover partially locked onto the pipe using the cam locking mechanisms, and from 15.25" at 102.5 psi (gage) without locking the cover. After each trial, the surrogate was closely examined, photographs were then taken to document the head model damage, measurements were made when applicable, and observations and data were documented.

For the test with a partially locked cover, air pressure was not sufficient to overcome the locking mechanism and no launch resulted. The long-range impact with the loosely-fitted cam-lock yielded only a small, hair-line fracture of the head form skull. The short-range and higher pressure test resulted in a significant fracture on the head form with a maximum crack length measuring about four inches from the impact site to the end of the crack, and three resulting skull surrogate fragments, with a combined size of about 1.18" x 1.57". When morphology of the cracks and broken pieces from this trial were subjectively compared with photographs of the accident evidence, similarities were observed, suggesting similar impact conditions. Evidence from the accident, however, consisted of only a single fragment measuring approximately 1.5" x 2". This difference in skull fracture pattern may imply different experimental impact conditions, but also may indicate the inability of the surrogate to accurately reproduce human skull failure modes due to different geometry or material properties. Because correlation of experimental results with the real-life event was largely subjective, further study on the subject should include additional research to simulate the accident more precisely, high speed videography to document the ballistic impacts, a complete "skin-skull-brain" model as a more biofidelic surrogate, and laboratory study to validate the "skin-skull-brain" model biofidelity as compared to controlled studies using postmortem human subjects.

Reference:

1. Thali, Michael J. , Beat P. Kneubuehl, Richard Dirnhofer "A "skin–skull–brain model" for the biomechanical reconstruction of blunt forces to the human head" Forensic Science International, Volume 125, Issues 2-3, 18 February 2002, Pages 195-200

Thali, Skull, Surrogate

D45 Velocity and Location Effects on Bruises Created Using a Controlled Ballistic Elastic-Mass Delivery System

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After attending this presentation, attendees will be educated on the effects of ballistic impact velocity and anatomical location on the immediate onset, size, duration, and color of bruises created on the thorax and abdomen of volunteers.

This presentation will impact the forensic science community by using bruising biomechanics to assist emergency room physicians in determining cases of possible physical abuse and forensic investigators in estimating the timing of recent blunt-impact injuries.

Existing literature contains conflicting information regarding the color changes in bruises with respect to time. There is general agreement that the first colors seen are usually deeper reds, black, and blue. Yellow is generally not seen for the first 18 – 24 hours, but after that, any color may be present. It follows that deeper tissue bruises, from higher-velocity impacts, will remain darker, longer. It is hypothesized that if mass is constant, a bruise from a slower impact will resolve more rapidly, have a smaller area with color change, and include lighter colors.

The relationship between velocity and bruise characteristics has not been thoroughly explored in published literature. Specifically of interest is the effect that impact velocity has on the affected area of color change (as measured at the point of impact and at 10, 20, and 30mm from the point of impact) as well as the magnitude of the color change. The effect of bruise location was also investigated.

Forty healthy adult volunteers completed an initial questionnaire to screen for bleeding disorders and blood thinning medications. Informed consent was garnered, and each subject had their thoracic and abdominal regions digitally photographed (Canon EOS 30D, Lake Success, NY). Prior to bruise creation, tri-stimulus light reflectance was also measured using a commercially available colorimeter (model CR-400, Konica Minolta) tri-stimulus light reflectance was reported using the three-dimensional CIE 1976 (L*, a*, b*) color space, where L* represents luminance (0 = black, 100 = white), a* represents the shift from magenta to green (green is indicated with negative values, magenta with positive values), and b* represents the shift from yellow to blue (blue is indicated with negative values, yellow with positive values). Changes in the CIE 1976 color space are represented by ΔL*, Δa* and Δb*. The overall change in the CIE 1976 space is characterized by the value ΔE, which is the vector sum or resultant of the three color space change variables ΔL*, Δa*, and Δb*.

Blunt impact injuries were inflicted using a pneumatically-fired system. The impacting projectile was a standard .68 caliber paintball, spherical in geometry with a mass of 3.1 grams. Impact velocities were either approximately 200ft/sec or 300ft/sec and impact energies were 5.75 J and 12.95 J respectively. Both body regions on each subject received impacts of the same velocity for 29 of the subjects, while 11 subjects chose to stop or the gun malfunctioned for one of the shots. The first shot location was varied randomly between two body regions and the shot velocity was varied randomly between subjects. Baseline measurements at both impact sites were compared to measurements taken immediately after impact, as well as 60, 90, and 120 minutes post impact. These measurements were taken at the point of impact and at 10, 20, and 30mm from the point of impact.

A repeated-measures analysis was performed for the 29 subjects that were impacted in both locations (SPSS version 19, IMB). A within-subjects comparison included the ΔE differences at: each body region, all four distances from impact, and all four time periods; and a between-subjects analysis was performed for the impact velocity. The color change was significantly greater at the higher impact velocity $F(1, 27) = 31.4$, $p < .05$. There was no significant difference in the color changes measured at the thorax compared to the abdomen $F(1,27) = 0.949$, $p = .339$. There was a greater color change immediately after impact when compared to all other times $F(1, 27) = 49.151$, $p < .05$. The color change decreased significantly at each increment of distance away from the point of impact $F(3, 25) = 50.697$, $p < .05$.

Bruising, Velocity, Quantification

D46 Atypical Postmortem Dental Identification

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After attending this presentation, attendees will possess a greater understanding of exceptional, non-traditional methods of postmortem dental identification when antemortem dental records do not exist or are otherwise non-retrievable.

This presentation will impact the forensic science community by emphasizing the areas of medicolegal death investigation, forensic pathology, forensic anthropology, and forensic consulting. In each of three cases discussed, human identity was confirmed within medical/legal certainty, utilizing an alliance of forensic dental autopsy and antemortem medical records or differential non-radiographic maxillofacial oral anatomy. With each case example, a greater knowledge of the scope and advantage of contemporary forensic odontology as an augment to traditional death and identity investigation will be gained.

Reasons that a forensic dental comparison is an ideal instrument in death investigation include the longevity and durability of the dental and oral anatomy, even in scenarios of extreme heat, trauma, decomposition, or a combination of all of these factors. Postmortem identification based on dental record comparison is universally recognized and consistently accurate. The reality however, that despite thorough investigation, antemortem dental records are not always recovered is addressed in this presentation. The alternative investigative methods employed that resulted in a positive dental identification, without antemortem dental records will be detailed.

The first case involved a white male seen in a parking lot pacing around a vehicle. Moments later, witnesses observed the vehicle engulfed in flames with the male sitting in the passenger seat. Emergency personnel responded and confirmed the death without intervention due to the thermal injuries. Homicide detectives were summoned to the scene and initiated an investigation. Autopsy confirmed the cause of death as inhalation of products of combustion and the manner of death as suicide. Antemortem dental records of the decedent were not recovered; however, medical radiographs of the head and neck were available. A forensic odontologist was summoned and antemortem digital radiographs, a frontal and lateral skull series highlighting a medical implant on the cervical vertebra, were offered for analysis. The dentition was visible on both radiographs and, because of the radiographic orientation, the lateral film showed the lower teeth clearer and with more detail. Tooth numbers 30 and 31 were restored with significant and unique dental restorations. Postmortem dental radiographs were taken and a positive identification was rendered based on the comparison of these dental restorations.

The second case involved a Hispanic male transient. 911 was called after a witness found the decedent floating in a creek. Paramedics arrived, recovered the body, and confirmed death. Autopsy determined the cause of death as atherosclerotic coronary artery disease with contributing factors of drowning and chronic alcohol abuse; the manner of death was accidental. The investigator summoned a forensic odontologist to complete a dental identification, yet no antemortem dental records were recovered. However, very recent frontal and lateral CT scans were collected and given to the odontologist for analysis. Postmortem dental radiographs were taken and a positive dental identification was rendered after precise antemortem image improvement to allow for a strict tooth-by-tooth comparison of the decedent's restored teeth.

The third case involved a motor vehicle collision resulting in two fatalities, a male youth and an elderly female. The bodies of both

decedents were charred beyond recognition and identification by dental record comparison was ordered by the medical examiner for both victims. Antemortem dental records for the female victim were quickly obtained and her identification was made without difficulty. The investigator learned that the youth was undergoing orthodontic treatment in Tijuana, Mexico, and it was assumed adequate dental records would be readily available for comparison; however, this assumption was wrong. No pre-orthodontic dental radiographs of any kind had been created, and only study models of the decedent's teeth, prior to orthodontic appliance placement, were available. Even with the extent of external charring, the oral cavity, dental, and palatal anatomy of the decedent remained unharmed. Dental impressions were collected on the decedent and models created from these impressions for comparison with the antemortem models. Although the tooth positions were now inconsistent due to orthodontic repositioning, the distinctive pattern of the palatal rugae had numerous consistencies and there were no unexplainable inconsistencies upon direct comparison. A positive identification was rendered based upon this comparison.

Investigation, Dental, Identification

D47 Ready for the Olympics? Disaster Victim Identification Training and Exercises as Part of a Capacity Building Process for UK Police Forces

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After attending this presentation, attendees will understand the educational philosophy and practical implications of uses of experiential learning for training and exercising in Disaster Victim Identification (DVI). Attendees will learn how the creation of scenarios that replicate, as far as is possible, key elements of a mass fatality incident will enable responders to develop the necessary skills in a controlled and educational environment.

This presentation will impact the forensic science community by presenting a systematic educational philosophy applied in police DVI exercises designed to prepare United Kingdom (UK) police forces for the London Olympics 2012.

Introduction: Following the Asian tsunami and large scale multi-centre terrorist attacks such as 9/11 and the London bombings of July 2005, DVI preparedness has been reviewed in many countries. Such reviews have covered various aspects of DVI, including team composition, local, regional, and national organization systems, inter- and intra-agency communication and co-operation, together with training and exercising.

DVI team members are drawn from a wide range of disciplines and can be broadly divided into two elements: (1) the scientific team members, such as forensic pathologists, anthropologists, odontologists, radiographers, mortuary technologists, laboratory officers, etc.; and (2) police officers or other emergency service responders. Whatever the structure and composition, it is essential that the DVI team members should be adequately trained and should have taken part in regular and comprehensive multidisciplinary exercises.

All team members typically perform their normal day-to-day role within a controlled environment. They will work with other professionals who understand their role and contribution to routine investigation. In a mass fatality incident, this will not be so; the situation will be unfamiliar and traumatic. Team members will need to act with confidence and speed and be mindful of the situation unfolding around them. The use of simulation training in a true multidisciplinary team environment helps team members gain first-hand experience of a realistic mass fatality situation, to plan and to understand the implications and limitations of their actions, and develop the necessary skills for disaster response within a controlled situation. By creating a simulated mass fatality incident in which all the elements of emergency forensic response are represented, team members can experience first-hand the multi-faceted challenges presented by such a situation. They can develop and try out their own strategies for overcoming practical and organizational challenges in a learning environment, supported by a team of experienced tutors. By use of multi-disciplinary training exercises, students gain understanding of the challenges faced by other professionals and learn to adopt a team approach to solving practical problems to achieve a common objective.

The Inforce Foundation has systematically applied the educational philosophy of experiential learning to a system of exercising that uses low-tech simulations in a number of different scenarios, ranging from mass grave excavations to mass fatality incident mortuary operations.

This presentation will concentrate on a series of simulation exercises that Inforce carried out with a number of UK police forces over the past four years. Most of these police hosted various aspects of the Olympic Games 2012. The exercises were designed to be flexible enough fit the precise needs of each force in question and work with their already existing level of qualifications and experience. By taking account of these variables, each exercise was tailored in terms of role distribution, rotation, difficulty level, and the elements of DVI operations to be included.

Results: Through these exercises, team members learned to:

- Understand the scope of a mass fatality incident.
- Adapt to changing circumstances.
- Develop the confidence to perform in different roles in the team.
- Contribute effectively to the team effort.
- Have consideration for the safety of self and others.
- Develop the confidence and skills to train other team members.
- Participate in the identification process.

Conclusion: There is no way to prepare adequately for a mass fatality incident as each and every incident will be different. However, such simulation exercises assist team members to prepare for, and adapt to, any situation as it unfolds, and to act professionally and confidently as part of a multidisciplinary team.

Capacity Building, DVI Exercise, Mass Fatality Incident

D48 Bone and Body Part Deposition in Rivers: Where to Look for the Rest of the Body

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After attending this presentation, attendees will learn the results of eight empirical trials performed by seeding rivers with real and synthetic bones and tracking where samples were deposited. Trends in the location (geomorphology) and river bed features at locations of deposition will be described.

This presentation will impact the forensic science community by learning about the most likely locations of deposition, which can aid law enforcement agencies in the recovery of more remains from fluvial systems, allowing search personnel to be deployed more efficiently to spend more time searching in locations with a higher likelihood of success.

Knowing the most likely locations of deposition can aid law enforcement agencies in the recovery of more remains from fluvial systems. Search personnel can be deployed more efficiently and can spend more time searching in locations with a higher likelihood of success, thus increasing the recovery rate of remains and reducing the cost of searches. In addition, since rivers are hazardous environments, personnel above and below water will be exposed to fewer hazards since searches can be targeted to higher probability locations, thus reducing the time individuals spend in and around dangerous working conditions.

To determine where skeletal remains are deposited in rivers, animal bones and casts of sheep bones were seeded in three rivers and observed yearly. Bones were seeded in the East Fork Sevier River (EFSR), UT, and Big Beef Creek (BBC), WA, and bone casts were

seeded in both these rivers and Levelock Creek (LC), AK. The EFSR is a sinuous river with a sand to gravel bed with a study reach of ~25 miles long, while BBC is a braided river with a gravel to cobble bed and a study reach of about two miles in length. LC is a highly sinuous river with a sand to fine gravel bed and a study reach length of ~1.5 miles. Empirical seeding trials were performed by placing bones or bone casts in the rivers with known initial locations and orientations then visiting the rivers every summer thereafter. During trial observations, the rivers were searched and, when samples were located, their location of deposition (geomorphology), bed characteristics (grain size, distribution, woody debris, etc.), distance downstream (measured with a fiberglass tape measure), orientation, and burial were recorded. Located samples were collected and archived for later laboratory analysis. A total of eight empirical trials utilizing bones are in progress, six in the EFSR, and two in BBC. Five empirical trials using bone casts are in progress, one in LC, three in the EFSR, and one in BBC. In addition to empirical trials, 14 rivers have been searched for skeletal material, and when remains were located, observations were recorded like the empirical trials.

Of the 6,000+ bones and 3,686 bone casts seeded, 218 bones and 307 bone casts have been recovered. While observing bones in rivers, 485 bones have been observed in addition to 33 articulated body parts. Of the 644 bones with good location descriptions, 4.3% (N=28) were found on point bars, 23.6% (N=152) on lateral bars, and 4.3% (N=28) on median bars, for a total of 32.2% of bones found on bars of one form or another. 26.4% (N=170) of bones were found on either the right or left banks, and the remaining 41.3% (N=266) were found in the thalweg (deepest part of the river channel). 24.7% (N=184) of bones were found in association with woody debris out of 744 bones with notes on woody debris associations, and three bones were found hanging in vegetation above the river bed. Results for the 307 casts recovered were broadly similar with 11.7% (N=36) found on point bars, 14.0% (N=43) on lateral bars, and 9.1% (N=28) found on median bars, for a total of 34.9% of bone casts found on bars. 33.9% (N=104) of casts were found on banks, and 31.3% (N=96) of casts were found in the thalweg. 53.1% (N=163) of casts were found in association with woody debris.

These results suggest that skeletal material can be found in any location with a change in flow competence (the ability of the river to transport material). This includes locations where rivers suddenly become deeper or shallower, like bars of any form (point, lateral, and median bars), as well as the upstream end of pools. In addition, flow obstructions (woody debris, bridge pilings, large rocks, etc.) catch skeletal material and prevent further transport, which accounted for many of the bones and casts found in thalwegs. Articulated remains showed similar trends though a large enough sample size for comparison is not yet available.

Taphonomy, Fluvial, Bones

D49 Missing Persons or Disposable Persons? Perils and Pitfalls in the Investigation of Unidentified Bodies

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After attending this presentation, attendees will learn of the methods in attempting to identify skeletal remains and also of the obstacles faced when working on a cold case with multiple unidentified victims.

This presentation will impact the forensic science community by familiarizing attendees with steps that can be taken in an attempt to identify victims in a cold case, and discussing the delays that can occur when wrong theories are made.

This presentation will review the efforts made to identify four homicide victims, a woman and child, then two additional children, whose skeletal remains were found in two separate barrels approximately 100 yards apart, in a forest in New Hampshire in November 1985 and May 2000, and the erroneous assumptions that were made regarding several aspects of the investigation. Presumptions regarding the value of the DNA profiles that were obtained from the victims, the usefulness of additional scientific testing, the interest of media organizations, and the capacity of technology to match missing persons to unidentified remains were not accurate. This presentation will also highlight the numerous government and non-government agencies and resources that can aid a medicolegal jurisdiction working to give a name to unidentified remains.

After attending this presentation, attendees will understand some of the challenges associated with reopening a cold case, in which the crime occurred and the bodies were discovered prior to the founding of the State Medical Examiner's office and prior to the use of computers to track evidence and store information. Difficulty locating all the skeletal remains from the four victims was encountered due to a variety of bones being sent to different labs, agencies, and authorities for analysis.

The limitations of utilizing mtDNA test results for identification purposes will be discussed, including that mtDNA profiles are not used in criminal databases, the inability to confirm there is a relationship between victims with matching mtDNA profiles, and that DNA profiles of unidentified remains cannot be compared to DNA profiles of known criminals to search for a familial match.

Scientific testing of these remains resumed within the past few years. A hair analysis of the adult victim was performed in an effort to determine what part of the country she had been in during the months preceding her death. The results were inconclusive.

Participants at this presentation will learn that, while a homicide case involving four victims, three of them children, would seem compelling in terms of public interest, unidentified remains are not as noteworthy to the media as cases of missing persons. Efforts to publicize New Hampshire's cold case have been statewide but were not picked up on the national level. While missing persons have families and friends who use the media in an attempt to find their loved ones, unidentified remains have no such advocates.

There are several websites that contain databases for missing persons and unidentified remains and New Hampshire's cold case is listed in some of these, including the National Missing and Unidentified Persons System (NamUs), a national resource for the records of missing persons and unidentified remains. It was originally believed these databases were capable of matching missing person cases to unidentified remains and that the medical examiner's office would be alerted to any possible matches of the New Hampshire victims to missing persons. It was later learned the databases are not capable of making matches and the missing person section of a database must be searched manually in order to find potential matches to unidentified remains.

Unidentified, Remains, Cold

D50 John Doe — Identified Seven Years Later: A NamUs Success Story

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After attending this presentation, attendees will learn about the National Missing and Unidentified Persons System (NamUs) and the successful 2012 identification of a John Doe who was found in 2005. At the conclusion of this presentation, attendees will be able to discuss the importance of submitting information from unidentified persons into NamUs.

This presentation will impact the forensic science community by highlighting the need for medical examiners, coroners, and death investigators to input information regarding unidentified human remains into NamUs. This should result in an increased participation of this system, which should then result in an increased success rate of identifying previously unidentified human remains.

The National Institute of Justice (NIJ) reports that the nation's medical examiners and coroners have an estimated 40,000 unidentified human remains cases, the majority of which have been buried or

cremated before being identified. This has been named the greatest mass disaster of our time. To remedy the problem, NIJ developed NamUs, a central reporting system for unidentified human remains that is inclusive, intuitive and open to the public. NamUs is structured to allow searches for matches between missing persons and information on unidentified human remains such as skeletal profiles, dental charts, fingerprints, DNA profiles and unique scene evidence. The database also matches identified unclaimed remains with individuals reported missing within the National Missing Persons database. Those agencies whose population includes high numbers of transient individuals (vacationers, students, migrant workers, etc.) understand that casework often crosses state lines and these groups will benefit from the nationwide approach to NamUs, as highlighted in this presentation.

In 2011, the Charleston County Coroner's Office was awarded grant funds for their "Bones in Boxes" Unidentified Human Remains Project from NIJ's "Using DNA Technology to Identify the Missing" initiative. As a result of those funds, a forensic anthropologist and forensic odontologist were hired to work on cold cases involving unidentified human remains. A case study of a "John Doe" who was found in 2005 in Charleston County will be presented. At that time, investigators used many methods to try to identify the decedent without success. The case was reevaluated in 2011 and a DNA sample was submitted to the University of North Texas for analysis. The DNA profile information was then uploaded into Combines DNA Index System (CODIS) and then into State DNA Identification System (SDIS). It was then entered into National DNA Index System (NDIS), where a positive match was made after comparing the DNA to samples uploaded into the Georgia Bureau of Investigation (GBI) convicted offender DNA database. The lessons learned from this cold case, including policy changes which were implemented in the coroner's office will be discussed. The ensuing investigation, the working relationship with out-of-state agencies to confirm the decedent's identity, and the notification procedure to the legal next of kin will also be discussed.

The Charleston County Coroner's Office has subsequently been entering all data on unidentified human remains and those cases in which the remains have been identified but are unclaimed. The overall results of their grant project, including the number of remains submitted for analysis and inclusion into the NamUs system, the number of remains identified, the number of remains that have since been claimed, and other lessons learned will be discussed.

NamUs, Unidentified Remains, Identification

D51 Forensic Podiatry: Importance of Foot and Footprints in Forensic Casework

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After attending this presentation, attendees will be exposed to an emerging discipline of forensic science, forensic podiatry, with an emphasis on estimation of stature from foot and footprints, and individual characteristics of footprints.

This presentation will impact the forensic science community by presenting a newer discipline of forensic science called forensic podiatry, which may motivate and inspire young forensic scientists to study this technique.

Forensic podiatry is a comparatively new scientific sub-discipline of forensic science. It is defined as the application of sound and researched podiatric 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. One of the main tasks of forensic podiatrists is to contribute to the establishment of personal identity in forensic investigations. The need to establish the identity of dismembered remains may arise in cases of mass disasters like terrorist attacks, mass murders, transport accidents, tsunamis, floods, and earthquakes. Furthermore, forensic podiatrists help in the analysis of footprints generally recovered at the crime scene.

The human foot has been in the focus for a variety of reasons in the past for detailed study of diabetic feet, for orthopedic reasons, for anatomical purposes, study of feet by foot and shoe industries and Army, and the most important is forensic study of the foot. Now, it has been universally accepted that a mature foot and its impression are not only unique to an individual but also provides highly valuable clues regarding personal identity. Human feet separated from the body are usually recovered at the scene of mass disasters, both man-made and natural. Footprints can be found as a kind of evidence and can be collected from almost all types of crime scenes. A human foot and footprints can provide clues for personal identification in three ways, i.e., by reconstruction of body size (estimation of stature and body weight) from different segments of the foot and footprints, sex determination from dimensions of the foot and footprint, and by individualistic characteristics of the foot and footprints. Estimation of stature from the foot and footprints is based upon the fact that, like other parts of the human body, the foot also has a definite and positive relationship with stature of a person. Estimation of stature is an important parameter in forensic investigation and is considered as one of the "big fours" of forensic anthropology. Stature, age, sex, and ancestry facilitate the narrowing down of the pool of possible victim matches in the forensic investigation process and help in establishing identification of the individual.

The individualistic characteristics like corns, pits, ridges, humps, creases, deformity, an extra toe, etc. can be considered as useful forensic evidences in establishing personal identity. The presentation discusses, with examples, various methods of personal identification (e.g. estimation of stature, from individualistic characteristics) from the foot and footprint with reference to a study of North Indian population.

Forensic Podiatry, Stature Estimation, Individual Character

D52 Problematic and Perspectives of Child Abuse Investigation in Colombia

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After attending this presentation, attendees will become familiar with the legal problems of child abuse in Colombia and will understand suggestions for the criminal investigation in these cases.

This presentation will impact the forensic community by describing the difficulty of the legal classification of some behaviors related with child abuse in Colombia. Attendees will also learn about useful research directives in certain cases of maltreatment that include criminal investigative strategies, check lists of data that must be recovered in any child or adolescent maltreatment or abuse case.

Child abuse and maltreatment are defined as a series of deliberate actions and/or omissions that are carried out by adults (parents, relatives, caretakers), or other children or adolescents, that result in physical or emotional damages or the imminent risk of serious damage or death.

Nevertheless, within the Colombian penal code, the child abuse phenomenon is not clearly defined. It is according to the characteristics of each case, as well as the public prosecutor criteria, that a crime can be typified as a personal injury, abortion, kidnapping, torture, human trafficking, sexual assault, sexual abuse, nutritional nonattendance, incest, or domestic violence, among others. This last one is the most frequently used to process the case. An example of this situation is the statistical results emitted by the National Institute of Legal Medicine and Forensic Sciences, which demonstrated that in 2012 there were a considerable number of medical examinations in children and adolescents involving physical injuries within the domestic violence context, sexual forensic examinations in the same population, and autopsies in cases of child or adolescent homicide. Although it can be expected, this information does not emphasize the conditions of the crimes like the specific kind of child abuse, physical evidence related, or behavioral evidence of the aggressor that can be observed in victims.

In the same way, although there are some guides and protocols about forensic medical examination on physical injuries and sexual violence, both in adults and children, there is not any manual to guide

the criminal investigation of child abuse cases. The investigation is restricted to fulfillment of routine activities which do not help provide an integral understanding of the phenomenon, undertake the right judicial decision, and achieve an effective children protection.

Doing a review of the current situation of these cases and analyzing child abuse typology, widely described in scientific and forensic literature, certain directives and checklists within the criminal investigation are suggested in order to emphasize an interdisciplinary work and approach to the victim (family and social structure, socioeconomic context, medical background, stage of development, scholastic performance, etc.), the aggressor (maltreatment antecedents, drug abuse, labor situation, mental condition, educative level, relationships, criminal antecedent, etc.), the crime scene (characteristic of the place, suitable inspection, compilation of evidence, versions given by the victim and the aggressor correspondence, etc.), and other alternative sources of information (documents, professors, neighbors, relatives, civil servants of social services, medical personnel, etc.), applicable in cases of physical abuse, Münchausen by proxy syndrome, shaken baby syndrome, negligence, psychological maltreatment, institutional abuse, sexual violence, and homicide of children.

Child Abuse, Investigation, Checklist

D53 Forensic Analysis of the Mechanisms of Death Used in Homicides Against Women by Their Partner or Ex-Partner (Femicides) Committed in Spain From 1997–2009

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After attending this presentation, attendees will be introduced to the concept of Violence Against Women (VAW), a moral violence used by men against women, and how to identify the symptoms and develop strategies to prevent these kind of attacks.

This presentation will impact the forensic science community by raising awareness to common features to help identify cases of violence against women and evaluate them properly and to learn from the results to analyze risk factors and provide remedies for prevention.

Learning Objectives: Violence is usually approached in terms of results. Most of the studies try to establish differences among the distinct types of violence considering the injuries, circumstances around the aggression or the procedures used to commit the attack, but it is not enough to know the real differences among them and how forensic evaluation can help to prevent new aggression.

Violence Against Women (VAW) has different features when compared to other forms of violence. It is a "moral violence" used by men against women, especially in the context of a relationship, and that is constructed under cultural references. It means that there is a part of this violence that is considered "normal" and this feature affects the evolution of this violence, usually increasing the risk of new and more serious aggression and, even, homicide. These features must be known for the forensic exam (to evaluate and to identify and diagnose this type of violence) and to develop strategies to prevent these new possible attacks.

Hypothesis: Motivation and objectives of VAW murderers are different to other types of violence. These differences had to be present in the mechanisms and procedures used to commit the murder, and they have to give us some reference about the element (cognitive and emotional) involved in the crime.

Brief Synopsis of the Content and Summary of the Results: The Judicial Sentences from Spanish Courts and analyzed the different modus operandi used over these last years (1997 to 2009, the last year with available data) was studied. This information is based on the forensic report of the autopsy, and the study has focused around the following indicators:

- Mechanism and instrument used.
- To use a simple mechanism and instrument or more than one (simple or mixed aggression).
- Direct use of hands to kill.
- Degree of violence used during the process of murdering.
- Time of the day when the homicide was committed.

Using these indicators we got different significant conclusions for forensic science and other disciplines related to the assistance, evaluation, and analysis of Gender Based Violence:

- There is not a constant either common pattern in the mechanism of death used by men to kill their partner or ex-partner (women).
- The most common modus operandi is stabbing (in Spain there are many legal limits to access to firearms).
- Depending on the degree of violence, there exist two main groups related to the degree of violence used to commit the homicide: one is related with the number of stabs (stabbing mechanism) and hits (battering mechanism) used, and the other one is related to the use of the hands as a direct instrument to kill (mainly strangulation and suffocation). The analysis shows how the degree has evolved in time, increasing the level of violence during the last years.
- The most violent homicides usually are committed at night. This data suggests that the homicide in VAW is part of an accumulative process that starts before the circumstances that are finished in the murder

The results show that there are elements used in homicides of women within a couple's relationship that can help to identify this specific kind of violence, and that can be used to address and focus the investigation in this direction. This last possibility is very important if it is considered that in often many countries, and sometimes in all countries, these deaths (murders) are presented like suicides or accidents.

The results also give references to identify risk factors related to these murders, and provide some keys to develop strategies of prevention to stop the evolution of violence and the result of murder within relationships with Violence Against Women (VAW).

Statement of the Impact: Most of VAW cases happen within domestic atmosphere without witnesses, and many times it is difficult to investigate the circumstances related to the crime. Knowing some common features can help to identify these cases and to evaluate them properly.

VAW, Homicide, Domestic Violence

D54 Disabilities and Elderly Abuse: A Preliminary Forensic Approach

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The goal of this presentation is to raise awareness and to characterize elder abuse in persons with disabilities in Portugal.

This study will impact the forensic science community by promoting an earlier detection and diagnosis of elder abuse and giving better guidance for the implementation of prevention strategies.

The normal process of aging makes elders more vulnerable and fragile, and therefore more prone to poor health than the rest of population. As the dependence increases, demands on family caregivers or trained health or social workers become higher, bringing a huge socio-economic impact and stress to the families, thus facilitating an abusive environment. This fact, associated with physical and/or mental disabilities, increases even more the risk of elderly abuse. Literature studies that approach elderly abuse have only recently started to appear, and thus the association between disabilities and elderly abuse is still in a lead-off period of research. Reported cases of abuse evaluated at forensic medical services constitute only a small part of the real number of cases, but their analysis can bring us important knowledge regarding this problem. Since there are few studies in Portugal concerning this issue, the general aim of the present study is to contribute to a better characterization of elder abuse in persons with disabilities in this country.

A retrospective study was carried out at the National Institute of Legal Medicine and Forensic Sciences in the years 2010–2011 based on the forensic medical reports performed during this period. The selected sample includes alleged victims of abuse, over 64-years-old, and with physical and/or mental disabilities (n=42). A disabled person was considered as one without autonomy and with a permanent impairment rate over 70%. This number corresponds to 7.1% of the total of alleged elderly abuse victims observed at the same place and period.

The majority of the victims were: women (66.7%); retired (95.2%); married (71.4%); with physical disabilities (71.4%; e.g., motor disability), mental disabilities (14.3%; e.g., dementia), or both (14.3%; e.g., neurological disorders due to stroke). Average age was 77.6 years old. Women were mostly abused by their partners (46.4%) and men by their children (50%). The alleged offenders were mainly: males (61.9%); victim's relative (97.6%) – 54.2% victim's children; living with the victims (76.2%). In 19% abuse pointed reason was the offender's abusive alcohol consumption. Most of the reported cases were related with physical abuse (95.2%) consisting in kicking, slapping, punching, and pushing (76.2%). There were no lesions in 38.1% and in 97.6% lesions were mild. The elapsed time since the last offense and the forensic medical evaluation was superior to eight days in 31%. Abuse was recurrent in 59.5% of the cases.

Published data states that abuse is more frequent among people with high levels of dependence compared with older people not so dependent, leading one to consider that the number of cases obtained in this study is clearly underestimated and that they aren´t being detected or are underreported; actually, due to their lack of autonomy, these individuals are unable to disclose abuse, which also impairs their detection. Results corroborate some of the risk factors that are described in literature for victims (female; more than 74-years-old) and for offenders (male; victim's relative—especially children; alcohol misuse). Even without serious lesions (almost 100% of the cases) this kind of violence can have relevant physical and mental health consequences for elderly people, with socio-familial and financial repercussions, which must be considered in future studies. Therefore, it is of utmost importance to implement strategies to protect people with disabilities because this health condition increases both the risk of abuse and of nondisclosure. One of the preventive measures should be the implementation of a mandatory notification by the health care professionals to the competent entities at the time of discharge of physically or mentally impaired persons, since they completely disappear from the social and health services radar. Another measure should be creating temporary residencies for these persons in order to give the caregivers some resting time.

Elder, Abuse, Disability

D55 Combining Human Analysis With Human or Computerized Analysis to Reduce Classification Errors

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After attending this presentation, attendees will have a better understanding of how forensic analysts might decrease classification errors by complementing human, manual analysis of data with computerized analysis or with a second analysis done blindly by another human analyst. While polygraph data are used here, the concept and application applies to other disciplines that are dependent on human analysis, e.g., fingerprints or tool mark impressions.

This presentation will impact the forensic science community by challenging forensic analysts to investigate how the pairing of analyses increases the accuracy of professional opinions in their respective disciplines.

Introduction: For some time, forensic analysts have recognized the value of having a second analyst review their work. Traditionally, reviewers have served in a quality-assurance capacity and how their conclusions impact error rates is not well known. This study examined how two analyses could be combined to reduce errors.

Method: In this study, polygraph charts from 88 single-issue field polygraph examinations were independently analyzed by 10 human evaluators seeking proficiency certification. In each of these examinations, "ground truth" (i.e., the certainty of the examinee's veracity) had been confirmed by criteria typically used in published studies: 48 deceptive and 40 truthful. Each of the evaluators blind-analyzed the data in each case and made one of three classifications: truthful, deceptive, or inconclusive (unable to decide). An "inconclusive" occurs when the data are either insufficient for proper analysis or when the analyst believes a truthful or deceptive opinion is equally probable. Inconclusives are intended to provide a way to avoid errors and are generally resolved with re-testing and are therefore not counted as errors.

The polygraph data in each case were re-analyzed separately using two commercially available and regularly used computer algorithms for the analysis of polygraph data: White Star 2 and OSS-3. Both algorithms were run from within an updated version of the polygraph software that created the original data (Axciton Computerized Polygraph, version 9.2.0). Each reports the probability of truthfulness or deception. These computerized classifications were individually paired with the human evaluators' classifications. After pairing, the decision was reclassified to "inconclusive" when the two decisions disagreed (i.e., one concluded truthful; the other, deceptive). If the human evaluator did not make a decision of truthfulness or deception (i.e., the classification was inconclusive), the algorithm's classification was used. Otherwise, the human evaluator's classification did not change. Each of the 10 human evaluators' 88 decisions was, in this way, reclassified twice; first by pairing them with the White Star and then with OSS-3 analysis.

The proportion correct and erroneous were totaled for each examiner for each method, i.e., the original (human) classifications and those based on the pairing with each of the two computer algorithms as described above. The data were then analyzed to assess whether there were any differences between the human evaluators' original classifications and the two reclassifications.

Results: Pairing of the human and computer algorithm outcomes resulted in statistically significant decreases in the proportion of errors for truthful or deceptive examines, or both, with both algorithms.

Specifically, the original classifications for the 10 evaluators averaged 87% correct and 13 % errors (excluding inconclusive classifications). The OSS-3 and White Star 2 analyses resulted in 82% correct and 18% errors and 90% correct and 10% errors, respectively. The overall proportions of correct and erroneous OSS-3 and White Star classifications were not significantly different from the averages of the human evaluators. When paired with OSS-3, human classifications averaged 89% correct and 11% errors. When paired with White Star, classifications averaged 95% correct and 5% errors. More simply, errors decreased an average of 21% when paired with OSS-3 and 62% with White Star.

Discussion: This study supports the idea that "two heads are better than one," whether a human evaluator's decision is paired with another (blind) human evaluator or a computer analysis, when the goal is to reduce decision errors. While human analysts were not paired with human analysts, the algorithms appear to represent two average human evaluators, and the results provide support for the premise that when two agree in their conclusions, the likelihood their analyses will yield fewer errors increases.

Blind Analysis, Computer Analysis, Reducing Errors

D56 The "CSI Effect" in a High-Profile Animal Cruelty Prosecution

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After attending this presentation, attendees will understand the need for better training of police, veterinarians, and animal cruelty investigators in the application of forensic sciences to the investigation and prosecution of animal cruelty.

This presentation will impact the forensic community by helping professionals understand how animal cruelty cases can be weakened by the failure of law enforcement to recognize the seriousness of such crimes, improper application of forensic methods to animal crimes, and slow and inadequate response to crimes in which animals are victims.

Potential penalties for severe animal cruelty have increased substantially in recent years. Extreme animal cruelty can be prosecuted as a felony offense in 48 states. Juries have held investigators to comparably higher standards of forensic evidence in such cases. These expectations often do not reflect the training and resources available to those involved in such investigations. This disconnect can result in unsuccessful prosecutions. A recent case history illustrating these problems and review steps that can be and are being taken to address the issue in agencies around the country is presented.

In 2007, two Baltimore, MD, boys allegedly poured gasoline on a young female pitbull and set her on fire in broad daylight. She succumbed to her injuries several days after the attack. The case of this dog, named "Phoenix" by those who cared for her, sparked international response and led to the formation of a permanent Anti-Animal Abuse Commission as part of the Baltimore City government. The case involved a variety of forms of evidence, including eyewitness testimony, expert testimony from a Baltimore gang specialist, street view video footage, veterinary records, evidence of accelerants on suspect's clothing and the dog's collar, and more. However, much of the investigation was delayed or did not follow accepted protocols of evidence collection, storage, chain of custody, and analysis due to the lack of resources and lack of police and veterinary experience in dealing with forensic evidence in criminal cases involving animal cruelty. Potentially significant evidence was either ignored or mishandled. The defense was largely based on forensic shortcomings in the investigation. Despite these problems, the initial trial of the suspects ended in an 11-to-1 jury deadlock to convict. A subsequent retrial several months later based on essentially the same evidence resulted in unanimous acquittal. Both suspects were later charged with other crimes committed against people while out on bail, including attempted homicide.

The lessons learned from this case and the implications for the proper application of veterinary forensic sciences to animal abuse investigations will be reviewed. Animals in such cases are both victims and evidence. In most cases, veterinary staff place the highest priority on meeting the victim's medical needs, which can compromise the collection of evidence. However, such interests need not conflict. Likewise, police are often unfamiliar with the evidentiary value of common elements at an animal crime scene, including feces, urine, blood, fur, and trace evidence. Efforts are underway to enhance the training of all professionals involved in animal cruelty investigation to avoid such problems in the future.

CSI Effect, Animal Cruelty, Veterinary Forensics

D57 Use of Canines to Detect Dried Human Blood and Instrumental Methods for the Determination of Odor Profiles

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After attending this presentation, attendees will learn about the principles of odor detection by canines, particularly human blood detection. Attendees will also learn of the volatile organic compounds comprising this odor and the methods for extracting and analyzing these volatiles.

This presentation will impact the forensic science community by expanding the general knowledge base concerning the abilities of canines and their use in support of law enforcement investigative challenges that require trace determinations of the Volatile Organic Compounds (VOCs) that compose the odor of dried blood.

It is widely accepted that canines have an exceptional aptitude for locating objects of interest based on odor. Recently, the first known detector canine trained specifically to locate small quantities of human blood has been utilized to assist crime scene technicians in locating hard-to-find blood spots for subsequent DNA analysis. It was hypothesized that this will be the first research to show, experimentally, that a canine is capable of locating miniscule quantities of human blood.

The capability of the blood detection canine to locate small blood spots of varying ages was evaluated using a canine that had been trained solely on aged blood, and had not been previously tested or exposed to fresh blood. To prepare the samples for evaluation, approximately one mg of blood (two blood drops) was placed onto carpet squares. The blood on the carpet squares was allowed to age in an open environment for a set amount of time. The age of the blood samples used ranged from one to twelve weeks. The canine successfully located all blood samples with no false alerts. This was the first time that the canine's ability to locate extremely small quantities of aged blood was demonstrated in an experimental setting.

In another set of experiments, the ability of the canine to locate fresh, compared to aged-blood was assessed. Two sets of samples containing human blood, aged and fresh, were presented to the canine. The aged set contained fresh blood spiked onto gauze pads and aged for two weeks prior to testing. The fresh set contained fresh blood spiked onto gauze pads within two hours of testing. The different gauze pads were placed in perforated cans for the canine to search. The canine responded positively to the aged blood samples, but did not show interest in the fresh blood. This indicates a change in odor profile from fresh to aged (decomposed) blood.

For the instrumental analysis of the odor profile of dried human blood of various ages, blood was drawn from three human subjects, placed in open glass vials, and allowed to age for a given amount of time before analysis. The headspace was extracted using Solid Phase Microextraction (SPME) and was analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). The resulting odor profiles for each aged samples were compared. A unique group of VOCs were present in only the fresh sample, and there was a distinct change in odor signature from fresh blood to decomposed blood, occurring around Day 1 and Day 2. The VOCs detected on the first day represent the odor of fresh blood, while compounds detected after Day 1 represent the compounds that have evolved due to decomposition of the blood material, and the older samples show a continual change as the decomposition of the blood progresses.

In additional experiments, the odor signatures of dried human blood collected using several extraction methods in addition to SPME were

compared. Extraction methods included SPME with various fiber types, dynamic headspace sampling onto a sorbent tube and activated charcoal sampling. The extraction methods were compared based both on the compounds in the odor profiles as well as their precision. Based on the VOCs identified, it was observed that the extraction techniques do not necessarily yield similar results, yet instead may be considered complimentary extraction methods. To gain a better understanding of which of these compounds might be recognized by blood-specific canines, mixtures of compounds based on the odor profiles determined by each extraction method were created and presented to the blood detection canine in order to observe whether the canine would elicit a similar response to the selected blood VOC mixtures as to the actual blood.

Canines, VOCs, Blood

D58 Development of Latent Fingerprints on Brass Cartridge Casings: Survival of the Firing Process and Impact of Latent Print Development Using Acidified Hydrogen Peroxide on Forensic Firearms Examinations

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After attending this presentation, attendees will gain a better understanding of how often latent fingerprints deposited on brass cartridges survive the firing process and the extent to which latent print processing using acidified hydrogen peroxide interferes with forensic firearms examination.

This presentation will impact the forensic science community by demonstrating the survivability of latent fingerprints on brass cartridge cases after firing and discussing the extent to which latent print processing using acidified hydrogen peroxide interferes with forensic firearms examination.

Latent fingerprints developed on fired cartridge cases may serve as key pieces of evidence during forensic investigations; however, the success of developing latent fingerprints on fired cartridge cases has been a challenge for investigators due to the nature of the firing process. When fingerprints are placed on cartridge cases prior to or while loading of the weapon, there is a high probability they are destroyed due to the extreme temperatures and abrasive forces caused by the firing process. Despite these odds, other researchers have demonstrated that fingerprints, on occasion, do survive the firing process. Several methods for developing latent fingerprints on brass cartridge cases are available, which include cyanoacrylate ester fuming followed by Rhodamine 6G (CA/R6G) fluorescent dye stain and Acidified Hydrogen Peroxide (AHP).

While the majority of previous research has focused on identifying various techniques to develop latent fingerprints, very little research has evaluated the down-range effects of the development techniques to forensic firearm examinations. This is of particular interest with AHP since it is an irreversible reaction having the potential to corrode the brass and negatively interfere with the various impressions linking that cartridge case back to the weapon from which it was fired. The present study is separated into two phases. Phase I examines the survivability of latent fingerprints through the firing process, evaluates the development technique (CA/R6G, AHP, or CA/R6G-AHP) yielding the highest number of latent fingerprint impressions after firing, and the processing time required to develop fingerprints using AHP. Phase II examines whether and if so, the extent to which AHP may interfere with forensic firearm examinations at various processing durations.

For Phase I, the results indicate latent fingerprints deposited using the latent print matrix standards (both amino and eccrine) did survive the firing process; however, no latent prints deposited using the natural fingerprint matrix obtained from the study participant survived. Second, all three techniques were successful for developing latent fingerprints; however, AHP and CA/R6G-AHP were superior to just CA/R6G alone. Third, a maximum processing duration of 75 seconds should be observed when using AHP.

For Phase II, the results indicate firearms examiners considered all cartridge casings suitable for identification, but were able to differentiate whether a cartridge case had been processed. However, of those cartridge cases which had been processed, there was no statistical relationship between the processing technique nor the duration of processing and the level of degradation observed by the firearms examiners. Additionally, there was no statistical relationship of correlation values obtained using MATLAB of the images of the breach faces for each cartridge case before and after processing. These results warrant further research to better understand how the chemistry of fingerprint matrices, time, and normal environmental degradation of latent print residue will impact latent print development and its survival through the firing process. Additionally, further research is warranted to better understand the extent to which AHP processing may interfere with forensic firearms examinations using other types of ammunition and weapons.

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Cyanoacrylate-Ester, Hydrogen Peroxide, Fingerprint

D59 Evaluation and Validation of the SABRE Hand-Held Device for Pre- and Post-Blast Explosive Detection

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After attending the presentation, attendees will have an understanding for the use of an ion-mobility instrument in the detection of explosives and non-explosives.

The presentation will impact the forensic science community by providing a quick and easy technique for the detection and differentiation of explosives and possible false positive triggers.

Rapid detection of explosives is needed to provide real-time analysis of residues on suspected terrorists, explosive devices, and criminal offenders in military and domestic forensic settings. Instrumentation such as hand-held portable devices exists in the public market which identifies explosives with varying success. With an instrument capable of operating at a high level of accuracy and specification, personnel in theatre or security check points are capable of making informed decisions with minimal time delay. In addition, the device must be ruggedized for many different environmental conditions. The purpose of this study is to evaluate a portable hand-held device, the SABRE 5000, in the rapid identification of pre- and post-blast explosives for the defense and forensic communities. The SABRE 5000 is a recent update of its predecessor, the SABRE 4000, and utilizes Ion Mobility Spectrometry to identify the presence of explosives.

To evaluate the SABRE 5000 was conducted for pre- and post-blast explosives. For pre-blast explosives, 1,3,5-trinitroperhydro-1,3,5-triazine (RDX), Trinitrotoluene (TNT), Pentaerythritol Tetranitrate (PETN), Ammonium Nitrate (AN), and Potassium Nitrate (PN), samples were created in methanol at several concentrations from 1 ppb – 1000 ppm. Each sample at each concentration was analyzed in triplicate on the SABRE 5000 to determine its limit of detection for each explosive. Type I (false positive) and Type II (false negative) errors were calculated for each explosive as well as the sensitivity and specificity for the instrument.

In order to test for post-blast explosive samples, a test explosive consisting of an 8 gram mixture of a 60:40 TNT:PETN booster was carried out in two 55 gallon steel drums filled with saltwater. The

detonations were carried out at the surface level of the saltwater and submerged half-way below the surface. Various types of witness plates (i.e., metal plates, cardboard, aluminum foil, etc.) were included to simulate a water vessel that was attacked in a saltwater area. This would allow for analysis of PETN and TNT in this sort of environment. Samples were collected from the water surface, the water at the bottom of the water cavity, and various witness plates.

The instrument's effectiveness as a real-time analyzer was assessed using a Receiver Operating Characteristic (ROC) analysis, the area under the ROC curve, and the likelihood ratio based on the slope of the curve produced. The analysis provides a set of thresholds for a variety of explosives, such as TNT, PETN, RDX, and other nitrate salt-based explosives, thereby allowing a confidence level to be set for each explosive. The ease of use, portability, effectiveness, and durability of the IMS was assessed in this project. Explosive samples, particularly the post-blast samples, analyzed by the SABRE 5000 were verified using a gas chromatograph-mass spectrometer (GC/MS) to ensure accuracy of results. This validation study will attempt to show whether the SABRE 5000 is ready to be employed for use at airports, border patrol, as well as in theatres.

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IMS, Post-Blast Analysis, Explosives

D60 Evaluation of Ambient Pressure Ionization Mass Spectrometry Techniques for Routine Analysis of Explosives

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After attending this presentation, attendees will be able to identify several of the most common Ambient Pressure Ionization Mass Spectrometry (API-MS) techniques, and more specifically, their capabilities and practicalities for routine trace explosives analysis. Ease of analysis, limits of detections, and comparisons with techniques currently being used in casework will be discussed.

This presentation will impact the forensic science community by providing the forensic science community with a head-to-head comparison of API-MS techniques with current analytical techniques for trace explosives analysis. The analytical techniques which are commonly utilized in trace explosives analysis include: GC/MS, CI-GC/MS, and HPLC. All three of these techniques require sample extraction and lengthy sample runs. API-MS techniques, however, offer the ability to analyze a sample in just seconds and, in some circumstances, without sample preparation at all. Furthermore, initial studies have shown API-MS techniques to be as or more sensitive than the current analytical techniques applied to explosives analyses.

API-MS is a fairly new and rapidly evolving area of mass spectrometry. Since the development and publication on the Desorbtive Electrospray Ionization (DESI) source in 2006, numerous variations have been developed. Within the API-MS family there are several different applied principles off of which the techniques are based. Techniques such as DESI and sonic spray ionization take advantage of a charged solvent spray to bombard a sample and generate characteristic ions. Other techniques, such as Direct Analysis In Real Time (DART®) and Low Temperature Plasma (LTP), induce ionization via interaction between the sample and a plasma. A final group of API-MS sources utilizes lasers to ionize samples and includes methods such as Laser Ablation Electrospray Ionization (LAESI). Since there are different mechanisms being utilized in each of these categories of techniques, it is important to understand the benefits and drawbacks each method provides.

While a number of different studies have looked into the individual techniques for explosives analysis, this study provides a head-to-head comparison of several of these techniques including DART®, DESI, and LTP. In this study over ten explosives are analyzed from a number of different classes including nitroaromatics, cyclic nitros, straight-chain nitros, and peroxides. Using standards of known concentrations, optimized methods for analysis were developed for the API-MS techniques and compared to documented methods for CI-GC/MS and HPLC, which are currently employed in casework. In addition, limits of detection of the explosives were determined and compared. To date, it has been shown that limits of detection for analysis by DART® have been up to two orders of magnitude more sensitive than CI-GC/MS. Furthermore, the API-MS sources allow for the analysis and differentiation of peroxide explosives, which could not be accomplished using current techniques. Additional portions of this study examine the effects of different sample substrates on analysis, and the addition of dopants to create adducts and increase sensitivity. In the case of DART®, the presence of acetone has been shown to increase the sensitivity for RDX and similar compounds with no deleterious effects on the sensitivity to other explosives. The results of this study have led to the development and implementation of a method of analysis for trace explosives using DART®-MS at the USACIL. Additionally, this study will prove the viability of API-MS techniques as a screening technique for trace explosives analysis in criminal investigations.

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Explosive Analysis, API-MS, Chemical Analysis

D61 Investigating Forensic Science Laboratory as an Undergraduate Student

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After attending this presentation, attendees will: (1) learn basic laboratory exercises that can be used in an introductory forensic science laboratory college course; (2) be able to teach students simple methods used to analyze a variety of forensic evidence; and, (3) understand the comparison of students' learning objectives throughout the course from day one to the final exam.

This presentation will impact the forensic community by showing specific laboratory exercises being developed and offered to students in undergraduate forensic science programs and courses.

With all the popular criminal investigation shows on television, most universities have seen an increase in interest of forensic science among students. Because of this increased interest in college students to pursue a degree in forensic science, many universities have either started to offer a series of forensic courses, develop a minor in forensic science within another degree, or created an entire forensic science program. Whichever the case, students come into these types of courses and programs with a media understanding of what forensic science is. This sometimes results in students changing their viewpoint on what forensic science really is and if this type of career is the right choice for them. Universities with forensic science programs have realized that being an accredited program is an important aspect for incoming freshman and the surrounding forensic community. Ensuring all forensic science degrees offer the same type of coursework is reassuring for crime laboratory directors and the students. Over the past year, nine universities in the United States received Forensic Science Education Programs Accreditation Commission (FEPAC) accreditation bringing the total to 38 accredited programs. FEPAC guidelines do not require forensic laboratory coursework until students are well advanced in their studies, such as 300 or 400 level courses. General science introductory courses are required and it is assumed that students will gain a basic laboratory experience. The addition of an introductory forensic laboratory course will aid in the student basic knowledge and understanding of the role of the forensic scientist in the criminal justice system. The ability to have students be able to perform hands on laboratory exercises at an introductory level will help them better understand how forensic evidence is analyzed in a laboratory. This

would be a stand-alone laboratory course with no congruent lecture. This will allow students to focus on the science behind the forensic science. All types of evidence would be analyzed at a very basic level, including crime scene investigation, pattern recognition, physical matches, impression comparison, serology, blood spatter, DNA analysis, chemical examination with explosives, illicit drugs, and inks, as well as trace evidence with hair and fiber examinations. Current laboratory manuals are available, however, offer a more advanced examination of these types of evidence using instrumentation or needing to have an advanced knowledge of microscopy techniques. Development of a more basic forensic laboratory manual with exercises that are simple with no need of instrumental knowledge is needed. This will satisfy the need for students to engage in a forensic laboratory learning how forensic scientists identify, characterize, individualize, and compare evidence. Being at a more basic level, students will not need to have background knowledge of chemistry and biology to enroll in the course. All types of students will be able to participate in a basic general forensic science laboratory course and gain the same understanding and knowledge about what forensic science really is all about. Students will be surveyed at the beginning of the course about their background, including major and previously completed science courses and laboratories. Before and after each individual laboratory exercise, students will be questioned on the topic. This will show the increase in understanding of forensic laboratory analysis for each type of evidence. At the end of the course, students will be given a version of the introductory to forensic science lecture final; this will then be compared to the students only in the lecture format to discover if the same knowledge is attained on the material whether is a lecture setting or laboratory stand-alone course.

Forensic Education, Laboratory Exercises, Undergraduates

D62 The Need for Background Investigations of Forensic Students

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After attending this presentation, attendees will become aware of the widespread lack of vetting of forensic students in the United States. Attendees also will be aware of the opinions of forensic educators concerning requiring background investigations of forensic students prior to participation in their forensic education programs.

This presentation will impact the forensic science community by preparing students for forensic careers with the expectations that these students are required to meet less-stringent entrance standards than other criminal justice professionals and other client-centered professions.

Two-year, four-year, and graduate programs in forensic science have proliferated in the last decade. The Bureau of Labor Statistics predicts a 19% growth in the forensic science technician occupation between 2010 and 2020. Growth in demand is likely to stimulate additional growth of forensic science programs as universities and colleges struggle to increase enrollment. The National Academy of Sciences has called for standardizing and strengthening forensic science education programs in America. Forensic science programs can be no stronger than the individuals who are educated in those programs. The National Institute of Justice guidelines concerning qualifications for a career in forensic science indicate that entrants into the profession are likely to be required to meet the same background checks as law enforcement officers. The Forensic Science Education Programs Accreditation Commission (FEPAC) standards require that accredited forensic science programs inform students that they are likely to be required to undergo the same type of background check as would a law enforcement applicant.

Some associate and baccalaureate and many post-baccalaureate professional education programs require some form of background investigation of their student participants. Most of these programs educate for professions that deliver some form of client service, such as social work, nursing, pharmacology, etc. and require client contact during the educational program. The underlying assumption operative in these vetting requirements is that by virtue of participation in the education program, the student will assume a role in which the student could potentially harm others. While client contact is uncommon in forensic education programs by virtue of the student's participation in the forensic education program, the student develops specialized knowledge that would enable the student to potentially cause great harm with that knowledge.

Voluntary basic law enforcement education programs, in which students are not sponsored by a law enforcement organization, are available in community colleges of many states. Students in those programs typically complete all the requirements for initial entry into the law enforcement profession when hired by a law enforcement organization. Some of the content in those programs provides education and training in law enforcement techniques that are typically shielded from public disclosure laws. Those programs require various forms of background investigation of prospective students.

An online survey of forensic educators with two- and four-year colleges and universities was conducted. The sample frame for the survey was those undergraduate forensic education programs in the United States that are listed on the American Academy of Forensic Sciences website. Most respondents were directors of baccalaureate forensic education programs in public institutions. Almost all respondents reported that their institutions informed prospective forensic students that they would likely be required to undergo a background investigation similar to that used for screening law enforcement applicants. A majority of respondents agreed that their forensic students were aware of disqualifiers commonly used for screening law enforcement applicants. The only form of background investigation used by only a few of those institutions was a requirement for prospective students to provide letters of reference. Almost all respondents either had no opinion or disagreed with any required form of background investigation for prospective forensic students except a requirement for letters of reference. Almost half of the respondents agreed that undergoing a background investigation would help forensic students learn about their future professional and personal ethical obligations than would learning in the classroom about those standards.

Forensic, Student, Background

D63 Transformation: Leading Change and the Roles of the Advanced Practice Forensic Nurse

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The goals of this presentation are to demonstrate the roles of the advanced practice forensic nurse, provide examples of the various settings where the forensic nurse practices, describe advancements in forensic nursing education, and explore or demonstrate how interdisciplinary collaboration enhances outcomes in forensic settings.

This presentation will impact the forensic science community by demonstrating how nurses work in the collaborative role of a forensic nurse in the multidisciplinary investigative team. They provide insight as experts discriminating between intentional and unintentional injury in individuals and connect appropriate intervention models in clinical situations.

Injury continues to lead in cause of death and disability significantly impacting health care utilization and costs. Historically, medical forensic work was rooted in medical examiners in Europe, with forensic nursing beginning in the United Kingdom in psychiatric and correctional facilities. Expansion spread to North America and Australia in the roles of sexual assault nurse examiners and death investigators through on-the-job training and certificate programs supplementing basic coursework. Forensic nursing education today endorses the theoretical and scientific approach to injury analysis ascribing that intentional injuries are preventable, are predictable, and can be explained through surveillance data collection, syndromic surveillance, and modeling defining the

characteristics putting people at risk in order to optimize interventions with optimal outcomes.

In 2008, The Robert Wood Johnson Foundation (RWJF) and the Institute of Medicine (IOM) launched a two-year initiative to respond to the need to assess and transform the nursing profession with the purpose of producing a report that would make recommendations for an action-oriented blueprint for the future of nursing. Four key messages were noted throughout the deliberations culminating in the Institute of Medicine's (IOM) Report, "*The Future of Nursing: Leading Change, Advancing Health"* released in 2010. The committee developed four key messages:

1. Nurses should practice to the full extent of their education and training.
2. Nurses should achieve higher levels of education and training through an improved education system that promotes seamless academic progression.
3. Nurses should be full partners, with physicians and other health care professionals, in redesigning health care in the United States.
4. Effective workforce planning and policy making require better data collection and an improved information infrastructure (IOM).

Forensic nurses have established their place as experts through advanced education, rigorously conducting research, translating evidence-based principles to patient care, and finally, inter-professional collaboration as members of multidisciplinary teams. Expansion of forensic nurse roles is further enhanced by the broad expertise of clinical nurse specialists such as pediatric and psychiatric nurse practitioners, consultants, researchers, and educators. Universities proactively have heeded the call to attain parity with other professions in additional to expansion of existing programs addressing the IOM Report and the National Academy of Sciences Report, "*Strengthening Forensic Science in the United States: A Path Forward*."

Advanced education prepares forensic nurses to practice as: consultants to law practices, death investigators who may also serve on fatality review teams, child abuse and elder abuse experts, correctional specialists, forensic trauma practitioners, disaster responders for the living and identification team members for the dead, critical incident stress debrief team members, and forensic psychiatric clinicians, to name a few. Each of these roles requires an understanding of theory, scientific inquiry, applied physical science, biomechanics, and crime scene and evidence collection. Core competencies essential to practice provide the ability to describe and identify injury, access, interpret, use, and synthesize violence data, design and implement prevention activities, and manage injury prevention programs. Researchers and educators develop and disseminate information related to injury risk to the community, other professionals, key policy makers, and stimulate change through policy, enforcement, and education.

Role development of forensic nurses has grown through university curricula for the advanced practice clinician. Their work with victims, perpetrators, systems, and providers globally addresses a need where violence prevention models can impact outcomes in the reduction of violence.

Inter-Professional, Advanced Practice, Forensic Nurse

D64 No Family Left Alone: The Family Assistance Unit Within the Harris County Institute of Forensic Sciences

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After attending this presentation, attendees will be familiar with the current role of the Harris County Institute of Forensic Science as it pertains to family assistance. The recently implemented Family Assistance Unit will be described and current responsibilities of the program will be explained. Future goals for family assistance will also be expressed. This presentation will highlight the Harris County Institute of Forensic Sciences (HCIFS) development of the Family Assistance Unit and attempt to illustrate the need for improved family assistance services within all Medical Examiner's Systems.

This presentation will impact the forensic science community by contributing to understanding the challenges in assisting grieving family members and identifying the need for a specialized program to assist with family assistance services within medical examiner and coroner jurisdictions.

In January 2012, the Harris County Institute of Forensic Sciences initiated the Family Assistance Unit with the addition of a grant-funded Victim Assistance Specialist position. This unit functions to bridge the gap between medicolegal death investigation services and family assistance by providing support services and community resources to the surviving family members. These services include assisting the family on the scene, acute grief counseling, referrals to non-profit social service organizations, and notifications of death. As a grief counseling professional, the Victim Assistance Specialist is able to offer compassionate support, active listening, and identify appropriate resources for them throughout the grieving process. Having a single point of contact for the family members within our large multi-division agency allows for continuity of information regarding the status of the case.

The act of informing family members of a death requires a responsible, well-trained, and sensitive individual who is able to cope with this mutually traumatizing experience. Family members of deceased victims have a wide range of needs and reactions to the sudden and untimely death of their loved ones. Consequently, the individuals who deliver the death notifications and the manner in which they carry out this duty factor significantly in the immediate and subsequent grief response experienced by the family. Until recently, Forensic Investigators performed notifications of death via telephone when the family was not able to be notified at a scene or hospital. While in-person notifications are always preferred, time and distance can make this impractical in a jurisdiction as large as Harris County. In March 2012, HCIFS and the Family Assistance Unit entered into a partnership with area chaplaincy organizations to provide in-person notifications of death. The chaplains are trained by the Family Assistance Unit to deliver death notifications with accuracy, sensitivity, and respect for the deceased and their families.

Future goals of the Family Assistance Unit include extending the time frame and number of contacts provided to family for assistance. With the addition of more staffed positions, the Family Assistance Unit will also be able to attend a larger number of death scenes with the Forensic Investigator in order to provide in-person acute grief counseling to the family of the deceased. The Family Assistance Unit is also working to provide workshops to employees of the Institute of Forensic Sciences that will address issues of self care, debriefing, and coping skills.

The Family Assistance Unit within the Harris County Institute of Forensic Sciences is the first of its kind in the United States to offer extended services to families by chaplains and licensed social workers. These professionals add a depth of personal service that is often lacking in the typical scientific medicolegal environment, shifting the focus from management and care of the decedent to that of the surviving family members.

Medical Examiner, Grief, Family Assistance

D65 Camera Phone Photography and AFIS Sufficiency

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After attending this presentation attendees will: (1) be introduced to the possibility of camera phones being a viable mode of photograph documentation; (2) understand the limitations and difficulties when using

camera phones; and, (3) learn digital imaging standards followed by SWIGIT and the FBI.

This presentation will impact the forensic science community by adding to the knowledge of fingerprint impression evidence documentation through introducing the idea of using camera phones as a photography device.

Relevancy and validity of camera phone fingerprint impression evidence photography may be possible by testing capabilities of many different camera phones when photographing fingerprint impressions. Digital images must be proven authentic and relevant, which is why standards of operation procedures are often followed during documentation to prevent inadmissibility of evidence.[1] Discussing difficulties with the camera phone photography and reviewing digital imaging guidelines for AFIS sufficiency may help to further understand limitations of camera phones.

This study will add to the knowledge of forensic science and may introduce the possibility of camera phones as a method of fingerprint impression documentation at crime scenes, especially where transient evidence is present. As technology keeps progressing, modern cellular phones become more capable and are used to do things other than phone calls, such as taking good quality photographs. A few examples of cell phones with eight megapixel cameras are the iPhone 4s, Samsung Galaxy II, and the Droid X. The cameras that are in many cellular phones today are capable of high-detailed photographs, but whether or not the amount of detail is acceptable for AFIS database searches was the question of this study.

Camera phones used for the photographs were obtained through faculty, friends, and family. Camera phone megapixels ranged from 1.3Mp to 8Mp and were mounted on a pseudo tri-pod made from a ring stand and clamps. Fingerprints were made on porous, semi-porous, and non-porous surfaces, which were then dusted with black or magnetic powder for enhancement. Photographs were taken while mounted on the pseudo tri-pod and also hand held. When possible, photographs were taken flash on as well as flash off.

The photographs were then sent for AFIS review to Charles Morden, lab director of the Michigan State Police, to be assessed for AFIS sufficiency. Of 16 camera phones, 11 produced photographs that were admissible for AFIS. All the camera phones which were admissible were at least 3Mp and above. The camera phones that produced photographs that were inadmissible for AFIS were 2Mp and below.

Some difficulties or limitations that became apparent during the photography of the fingerprint impressions were camera phones that have no flash, camera phones with flash that drowned out detail, and proportion distortions from hand-held photographs. Camera phones that did not have flash in some instances had a difficult time focusing. Camera phones that did have flash often caused a reflection from the surface of the fingerprint impression to wash out the details of the photograph. The hand-held photographs had distortions that could be seen on the scale of the photograph. This is evidence that the camera was not parallel to the surface at the time the photograph was taken.

Standards for fingerprint impression digital imaging are stated in the guidelines of the National Institute of Standards and Technology as being 500 ppi (NIST 2011), but the standards from the Scientific Work Group Imaging Technology state a 1000 ppi guideline (SWGIT 2010).[2,3] This makes it hard to estimate a value of megapixels that would consistently produce AFIS quality photographs.

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AFIS, Fingerprints, Camera Phones

D66 19th-Century Jewish Cemetery in St. Maarten: Historic Facial Approximation Using Modern Technologies as a Doorway for Forensic Cases

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After attending this presentation, attendees will better understand the capabilities of modern facial reconstruction technology, as demonstrated utilizing a historic case example.

This presentation will impact the forensic science community by illustrating the results of working with multidisciplinary techniques and technology that lead to the ability to create modern 3D printed models. It will also help educate the forensic community about the current and future uses of 3D scanning and full-model 3D printing.

Facial approximations (reconstructions) are often used for medicolegal reasons, but they can also illuminate and give life to historic characters that in turn give rise to placement of historic communities in an appropriate context. Historians concur that the Isla de San Martin was discovered by Christopher Columbus on November 11, 1493 and claimed the island for Spain. For all intents and purposes, primarily Dutch and French settlers populated the Island of St. Maarten in the 1630s as Spain did not develop the island and allocated its resources elsewhere. The extent of the population of Jewish settlers was relatively unknown until the discovery of a Jewish cemetery. Historic research, anthropological methods, and, ultimately, a facial reconstruction give a face to one of the undocumented Jewish men buried at the forgotten cemetery location, and thus sheds new light on the contributions of this community to the islands economic and cultural development.

In 2010, a Jewish cemetery was discovered when research for other historical purposes was being performed. The discovery of a Jewish cemetery that was sold by the government and covered with residential houses was made. The Saint Maarten Archaeological Center (SIMARC) found remains of a decedent intact. A sample of dentition was sent to the College of William and Mary where a mitochondrial DNA analysis was performed. The remains were determined to be from the haplotype group U of Sephardic Jewish ancestry.

After an anthropological study that produced a biological profile, a facial reconstruction was made by creating a replica of the skull with photographs and measurements from the actual skull. A replica of the skull was created using silicone mold-making methods. With the replica of the skull a facial reconstruction was made with clay. State-of-the-art 3D scanning services were then donated by Engineering and Manufacturing Services (EMS), Inc. to 3D scan the reconstruction and then create a 3D resin print out of the reconstruction for museum use. A durable reconstruction was made to be placed for display at the Philipsburg Jubilee Library in Philipsburg, St. Maarten.

Ground Penetrating Radar (GPR) Systems were also used to map out the potential sites of the cemetery on nearby grounds to assist in the culmination of the documentation of the Jewish history in St. Maarten.

The ability to streamline the data using familiar methods in conjunction with modern technology has given the historical skeleton a proper placement in the community's history. These techniques demonstrate the positive results that international collaborative efforts could produce in forensic cases in the future. Issues such as preservation of chain of custody, release of materials, and other procedural concerns can be mitigated, as the original material may not ever need to leave its current locale.

Facial Approximation, 3D Laser Scanning, Historic Archaeology

D67 A Case of Manganese-Induced Parkinsonism: The Need for Safety in the Workplace

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Occupational exposure to manganese and the need for medical management of manganese-associated toxicity are well-established. After attending this presentation, attendees will understanding how both remain problematic in industrial settings.

This presentation will impact the forensic science community by illustrating the need to improve security rules in workplaces and to regularly inspect workplace healthiness in order to avoid possible exposure to toxic substances.

The case involved a welder who developed Manganese-Induced Parkinsonism after being exposed for twenty-six years. The welder worked in the steel industry, from the age of fifteen to forty-one years. His duties included arc and gas welding, using manganese-containing electrodes. This activity emitted welding fumes containing high levels of manganese.

At the age of thirty-six, the welder began to show extra-pyramidal rigidity, balance dysfunction, tremor at rest, bradykinesia, symmetric impairment, amimia, and "coq au pied."

Subsequently, psychiatric disorders, including major depression and manic depression-psychosis also appeared.

The welder underwent several clinical and instrumental examinations, including MRI brain scans, that indicated T1-weighted hyperintensive signal in the globus pallidus, bilateral.

These results, atypical of idiopathic Parkinson's Disease (PD), in association with the young age of the patient and his working activity, alerted the suspicion of Manganese-Induced Parkinsonism. Therefore, the welder had blood and urine tests which revealed high levels of manganese, confirming the work-related exposure.[1-6]

Instrumental research and laboratory tests, supported by clinical findings and circumstantial data, were essential to clarify the cause of extra-pyramidal symptoms.

Manganese is a heavy metal widely distributed in the environment by wind erosion and the manufacturing process. More than 90% is utilized in the manufacture of steel: it is alloyed with steel to increase strength, hardness, wear resistance, and with other metals to form highly ferromagnetic materials.

Manganese compounds are used as coloring, catalysts, oxidizers, and antiseptics. In biology, manganese is a trace element, a co-factor for a large variety of enzymes with many functions.[1,3,6]

Occupational exposure generally occurs by inhalation, then it is carried by the bloodstream to high-metabolism rate organs. Manganese half-life is approximately between 40 and 400 days and it is eliminated through fecal and bilious channels, a tiny amount by the urinary tract.

Toxic effects of manganese are related to its neurotropism; however, the basis for the selective neurotoxicity of manganese remains incompletely understood. It causes neuronal injury, especially in the globus pallidus, and also in other basal ganglia structures such as caudate and putamen, less frequently in the substantia nigra.

It primarily interferes with dopamine biology (the primary cellular energy metabolism), decreasing its receptors levels.[1,3,5,6]

An acute manganese-related disease is the metal-fume fever, resulting from inhalation of volatile metal oxides produced during welding or cutting of metal materials. The symptoms are generally nonspecific flu-like complaints including fever, fatigue, and muscle ache. Symptoms are self-limiting and typically resolved within 24 hours.[1,7,8]

Chronic manganese exposure produces an excessive accumulation of the metal in the brain that results in a neurological syndrome with cognitive, psychiatric, and movement abnormalities. The initial symptoms, manifesting within one to two years after the first exposure, are aspecific. Welders may also present neuropsychiatric disorders, popularly called "manganese madness." Over the long term, manganese-exposed workers exhibit an extrapyramidal syndrome that resembles PD: symptoms include gait disturbance ("coq au pied"), balance dysfunction, symmetric impairment, masked face, relative absence of tremor, grimace, bradykinesia, and muscular rigidity.[1,3,5,6]

MRI brain scans in manganese-intoxicated patients demonstrate a characteristic signal abnormality on T1-weighted studies that are not seen in normal individuals or in patients with PD or other forms of parkinsonism. Instrumental examinations show a hyperintensive signal, particularly in the globus pallidus. Similar changes are noted in the substantia nigra, caudate nucleus, and putamen.[5,6] Diagnosis is based on case history and neurological evaluation: in particular, hyperkinetic movements and the "coq au pied" step give evidence of a Manganese-Induced Parkinsonism rather than PD or Parkinsonism induced by other causes.

Prognosis is generally severe because neurological injuries are progressive and worsening, even after exposure cessation.[1,3,5,6]

In conclusion, this case study demonstrates the need for proactive interventions in order to improve workplace safety, minimizing manganese exposure and, therefore, preventing the onset of Manganese-Induced Parkinsonism. The disease is progressive and disabling, causing social disadvantages, especially tragic in view of the young age of onset.

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Manganese, Parkinson's Disease, Workplace Safety

D68 Misinterpretation and Inconclusive Medicolegal Evaluation Due to False Evidence Obtained From Non-Expert Crime Scene Investigations

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After attending this presentation, attendees will understand a criminal case in which a crime scene analysis, carried out by investigators without specific knowledge of the potentials offered by forensic botany, contributed to the loss of major probatory elements.

This presentation will impact the forensic science community by exposing the lack of a quality control system at crime scenes and to start a debate about the need to train specialists to ensure complete accuracy at the crime scene.

The dead body of a young woman was found on the sofa in her house in May 2012. Considering the lack of traumatic lesions, sudden death or death due to illicit drugs was hypothesized.

After a brief crime scene investigation, the body was transported to the Section of Legal Medicine for autopsy.

Death occurred due to heroin and cocaine overdose, as the toxicological analysis demonstrated. On the subjects clothes and inside the oral vestibule, the examiners found some botanical materials, identified by the botanical laboratory as dried flowers and perulae of *Laurus nobilis* and as ovary of *Wisteria sinensis.*[1,2] This evidence, along with some small nasal and forehead contusive lesions, suggested a fall in the external environment and successive shifting of the body by another person. Since the transfer of illicit drugs and death following another crime are two separate offenses in Italy, in overdose cases this behavior is often carried out to disguise the crime.

Therefore, a second crime scene analysis was performed. Examination of the garden revealed the presence of a *Laurus nobilis* and *Wisteria sinensis* trees and that the porch pavement was covered in flowers and fruits of these species (association between the victim and the scene).

Police officers stated that other clues, such as the woman's mobile phone, a beer bottle on the porch, and the strange position of the woman's body on the sofa (the body was covered by a blanket but her T-shirt was partially raised) indicated a secondary shifting.

However, they reported that the sheet used by undertakers to wrap the body was previously placed on the porch pavement. The highly probable contamination by plant remains occurred during such operation made the evidence collected on the body almost useless, as it could not be discriminated from the successive contamination. Thus, highly informative elements could not be taken into account.

This case is a serious example of how essential probatory elements can be damaged by non-properly trained investigators, lacking specific knowledge of the potentialities of various forensic sciences.

Although over the last century the forensic science community focused its attention especially on technological innovations, quality control systems, and accreditation of forensic laboratories, in recent years there is more interest in crime scene investigation as the pivot of the entire forensic examination and detective work.

Crime scene analysis is crucial to shelter the scene and to examine, assess, interpret, record, and collect physical evidence for court purposes, but also to direct the subsequent investigations, to establish priorities, and allocation of human and economic resources.[3,4]

Therefore, crime scene investigation can be considered a central part of forensic science, although not truly scientific, and not simply a technical discipline.[5,6]

However, in spite of the elaboration of a great number of procedural protocols, aimed at unifying the operative procedures and guaranteeing an objective approach to the scene, crime scene investigations are still far from a standardized quality control system and from a uniform organization (both in different countries and in the same one). Furthermore, performance indicators show an absolute link between outcomes and the knowledge and awareness of each investigator (the examiners tend to see only what they know).[7] For this reason, it seems to be necessary to establish minimal requirements for those attending the crime scene and to set up a new organizational model with a qualified person in charge.[4] This individual requires vast knowledge of the potentialities and limits of various forensic sciences and he/she must be able to carry out a correct and precise triage of the crime scene to identify its complexity and to possibly request the help of forensic specialists to ensure maximum quality in the gathering of evidence and in chain of custody.

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Quality, Triage, Training

D69 Recovery of Latent Fingerprints After Immersion in Various Aquatic Conditions

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After attending this presentation, attendees will understand the factors that affect latent fingerprint evidence submerged in a freshwater stream and the differences in latent print quality that can be recovered from various aquatic conditions.

This presentation will impact the forensic science community by providing results from an experiment that took place in a natural setting. There is limited published research on the topic of submerged evidence, and even fewer of these experiments utilize a natural aquatic environment. This presentation will add to the research being carried out in the field of underwater crime scene investigation and evidence preservation by broadening the understanding of how latent fingerprints submerged in water are affected by aquatic conditions such as current velocity, sediment types, and macrophyte interaction (i.e., plants, algae, and aquatic life). In addition, it will provide documented results of the deterioration of latent fingerprint evidence as the amount of time submerged in water increases.

As the use of waterways continues to increase for recreational purposes, so do the incidents of criminals using waterways to dispose of evidence.[1] Although some may believe that items recovered underwater will have no forensic value, this research shows that identifiable fingerprints may still be recovered. An experiment was conducted to establish the value of latent fingerprint evidence that had been submerged in a natural aquatic environment. The two factors analyzed in this study that affect the deterioration rate of latent fingerprints were stream current and length of time submerged. To evaluate these factors, latent fingerprints were deposited on metallic objects simulating the substrate of a knife or gun and submerged in a freshwater stream at locations subject to various powers of current for 24, 48, and 72 hours. After recovery, the items were subjected to cyanoacrylate fuming followed by black powder processing; the prints were lifted with tape and

examined. Each print was evaluated for its individualizing power based on a scoring system. Latent fingerprints subjected to higher current velocity were significantly more deteriorated than prints subjected to little to no current. A decrease in latent fingerprint visualization with longer periods of submersion was also observed.

It is known that the quality of latent fingerprints naturally deteriorate over time and this study supports this conclusion.[2-5] However, descriptions of the deterioration of friction ridge impressions on a metallic substrate submerged in a natural aquatic environment have not been well-documented and an evaluation of the environmental factors has not been thoroughly investigated. Whereas previous studies reported the successful recovery of good to very good quality latent fingerprints after seven days of submergence, this study has shown that after only three days in a natural aquatic environment, friction ridge impressions on a non-porous stainless steel surface were almost completely obliterated.[6] This illustrates the significance of the factors at play in nature. Studies conducted in an aquarium using only tap water, no current, and no macrophyte, or sediment interaction, are likely to produce deceptive results in comparison to studies conducted in a more natural environment.

In conclusion, the results of this study demonstrate the importance of understanding the interactions between latent fingerprints and the various factors of the natural aquatic environment to better aid in criminal investigations and potentially linking evidence to a perpetrator.

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Latent Fingerprints, Aquatic, Current Velocity

D70 Determinants of Turnover Intentions, Helping, and Knowledge Sharing in Crime Laboratories

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After attending this presentation, attendees will have a better understanding of reasons why crime lab workers choose to quit their jobs, gaining insight into managerial tools for reducing turnover and creating a more productive workforce.

This presentation will impact the forensic science community by providing the first known results from a study examining crime lab worker turnover, helping behaviors, and sharing of knowledge.

Forensic scientists are knowledge workers and are a laboratory's single greatest enduring expense. Therefore, it is imperative for forensic managers to find ways to retain employees, share knowledge, and create a cohesive, coherent team perspective. Based on a discussion with a group of FORESIGHT forensic laboratory directors in 2011, four major areas of research interest were identified: (1) reducing employee turnover; (2) increasing employees' helping behaviors with colleagues; (3) knowledge sharing among employees; and, (4) creating and disseminating a strategic vision to all employees.

Helping and knowledge sharing are also known as Organizational Citizenship Behaviors (OCBs). Job satisfaction and embeddedness would help explain turnover intentions and OCBs is surmised. It is very important to note that these two job-related constructs are very different. Whereas job satisfaction is the degree to which an employee is content with his/her job, job embeddedness refers to the psychological, social, and financial influences on employee retention and behavior. Strategic planning, vision, and employees' understanding of that plan are thought to be an important part of any organization's performance. Therefore, we would expect that employee understanding of the strategic vision would be positively associated with job satisfaction and embeddedness.

A 2000 review of the academic literature spanning the prior thirty years found job satisfaction to be the strongest predictor of employee turnover and desirable OCBs. More recently, researchers have found that employee embeddedness is an important predictor of turnover and OCBs, after controlling for job satisfaction. Therefore, we hypothesize that two direct predictor variables of turnover intentions and OCBs would be job satisfaction and embeddedness.

In addition to strategic vision, there are several other determinants of job satisfaction and embeddedness. Characteristics of the job, called work design, should have an effect on the forensic laboratory employee's degree of job satisfaction and embeddedness. Accordingly, we examine the job design attributes of autonomy, accountability, task significance, efficiency, and authentic leadership style. Autonomy refers to the amount of discretion a worker has in making decisions and scheduling tasks. Accountability suggests being held answerable for one's actions. Task significance is the extent to which employees believe their job impacts others' lives. Authentic leadership is a leadership style that subordinates see as motivational, just, transparent, and ethical. The hypothesis is that these attributes (along with strategic vision) will predict job satisfaction and embeddedness, which in turn predict turnover intentions, helping, and knowledge sharing.

Using the areas of research interest, a web-based survey containing all of the aforementioned constructs of interest was conducted. Five hundred and ninety participants from nine forensic labs in the United States and Canada participated in the survey. The statistical technique, structural equations modeling, to test the causality of the relationships discussed thus far was used.

Regarding turnover intentions, it was found that both job satisfaction and embeddedness were significant predictor variables. Job embeddedness was twice as strong as job satisfaction in predicting turnover intention. The same finding held true for predicting knowledge sharing. Job satisfaction was positively associated with helping behaviors.

Strategic vision was the strongest predictor of job embeddedness. Autonomy, accountability, task significance, and leadership style were also significant predictor variables. Autonomy was the strongest predictor of job satisfaction. Strategic vision, accountability, and task significance were also significant predictor variables.

The major findings from this study include the importance of job embeddedness, strategic vision, and autonomy in forensic laboratory employees. These variables, along with accountability, task significance, efficiency, and leadership style are manageable factors. To this end, the forensic laboratory manager offers practical ways to reduce turnover intentions and increase desirable OCBs.

Turnover, Knowledge Sharing, Helping

D71 ICAN and Adolescent Suicide Coroner/Medical Examiner Investigation Procedural Guide Implementation and Evaluation

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WITHDRAWN

D72 To Toss Film or Not to Toss Film? That is the Question in Radiography of Occult Fractures in Infants

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After attending this presentation, attendees will have a deeper understanding of key radiographic and technology-related considerations for the imaging of subtle pathology in infants using an animal model, also learning about the most and least appropriate approaches to such radiographic examinations.

This presentation will impact the forensic science community by reviewing traditional approaches to postmortem forensic imaging in infants while suggesting and validating alternate radiographic approaches in such cases.

The value of radiography in forensics is beyond question. However, employing radiography to document occult fractures in infants, less than one year old, can be challenging. In order to clearly visualize these subtle fractures, three factors must be taken into consideration; the focal spot size of the X-ray tube, the kilovoltage peak or kVp selected, and the image receptor.

The present study compared three imaging systems; a standard radiographic unit currently available in clinical settings, a dedicated mammographic unit that was no longer clinically compliant, and an X-ray cabinet unit. The largest focal spot, 0.6 x 1.2mm, was found in general radiography unit and the smallest, 0.1mm, with the cabinet X-ray system. The mammography unit had two focal spot sizes: 0.4mm for non-magnification imaging and 0.1 – 0.2mm when the magnification mode was selected. All images were recorded with either a digital, computed radiography or CR system and on X-ray film specifically intended for mammography. With film as the recording media, 30 – 40kVp was chosen to produce a high-contrast image. The kVp selection of 60 for the CR system was based on the manufacturer's guidelines for the minimum required to stimulate the receptor crystals within the imaging plate. However, in order for a more complete comparison, CR images were also taken at 40kV. The objective of the study was to determine which imaging system demonstrated a transverse fracture on the right third rib of a preserved fetal pig that had a crown-rump measurement of 29cm. A Visual Grading Analysis (VGA) methodology was employed to evaluate a total of 12 images and all images were reviewed by a radiologist, a radiologist assistant, and a senior radiographer, and compared to a reference image.

The image which scored best in terms of ability to resolve the fracture and related anatomy was acquired using the X-ray cabinet system at 35kVp and recorded on mammography film with a mean Image Quality Score (IQS) of 8.67. This was closely followed by the image acquired at 60kVp using the same combination of mammography film and the X-ray cabinet system (mean IQS = 7.33). However, the image obtained on film with the mammography unit set at 30kVp in the magnification mode clearly demonstrated the fracture despite a lower mean IQS of 1.67. The system receiving the lowest mean IQS was the general radiographic unit combined with mammography fil at 60kVp (mean IQS = -9.33) and 40 kVp (mean IQS = -10.0). Combining the general radiographic unit with CR at 60kVp resulted in a mean IQS of 3.33. Unexpectedly, the same system taken at 40kVp produced an image with similar performance (mean IQS = 5.0) to those acquired on film (mean IQS = 4.33). The manufacturer's recommendation for the higher kVp setting was intended to minimize the dose to the patient. However, in forensic imaging patient dose is not a consideration.

In conclusion, depending on the radiographic equipment that might be available, technical factors can be adjusted to demonstrate occult fractures.

Radiography, Infants, Techniques

D73 Canine Detection and Discrimination of Cadaveric Human Blood

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After attending this presentation, attendees will have a better understanding of how a well-trained detection dog team can maximize the collection of evidence from crime scene and improve investigative efforts

This presentation will impact the forensic science community by providing a variety of benefits to law enforcement regarding the need to improve the performance, reliability, and courtroom defensibility of detection dog teams and their optimized combination with both medical and forensic operators.

This study has determined that trained detection dogs do not alert to generic scent, rather they alert to blood traces, maximizing the location of blood trace evidence in an efficient, cost- and time-effective manner while minimizing the collection of samples not relevant to an investigation.

The scientific and medical evaluation of the crime scene requires a relatively short response time to avoid contamination of the same and, therefore, a well-trained dog allows faster coverage of very large areas of research, preserving any and all possible evidence.

The technique of shaping with positive reinforcements was used to obtain the result of blood scent detection and discrimination.

The goal of this research has been to identify and quantify the minimum mass of cadaveric blood (low concentration) required in order to potentially generate an alert by a detection canine by a *positive predictive value* (PPV).

Field trial experiments to determine canine interest in the observed blood samples were conducted.

The canine detection of blood scent in low concentration is called "*sensitivity.*"

In addition, this study reports the analysis of several potential interference odorant compounds at these blood scent traces in minimum concentration, and the associated percentages of false positive alerts (false PPV).

The canine discrimination of blood scent traces in minimum concentration is called "*specificity.*"

After training the detector canines to the cadaveric blood, a series of field trials are performed to test the canine's *"Limit Of Detection"*

(LOD) for the blood, which is the lowest quantity of a substance that the dog can distinguish from the absence of that substance (blank value).

The limit of detection (LOD) has been determined by performing scent line-ups in which various amounts of blood have been exposed and the lowest concentration of blood for which the canine can still alert have been recorded.

The study demonstrates that canines are generally not using the relatively low volatility parent substances, but instead use characteristics volatile headspace components to accurately locate specimen of blood, with the ability to distinguish between living and deceased human blood, as well as between human and animal blood.

Detection canines are the most common and widely accepted biological detectors due to the ability of canines to quickly and reliably locate the source of an odor to which they are trained.

The results of this study indicate that the well-trained cadaver dog is an outstanding tool for crime scene investigation displaying excellent sensitivity (88%), specificity (97%), and having a positive predictive value (94%), negative predictive value (98%) as well as accuracy (96%).

These recovery rates, ranged between 88% and 98%, indicate that properly trained cadaver dogs can make significant contributions in the location and recovery human cadaveric blood traces.

Canine Detection and Discrimination, Crime Scene, Cadaveric Human Blood

D74 Evaluation of Latent Fingerprint Development Techniques for Metallic Evidence

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After attending this presentation, attendees will understand the limitations currently associated with the development of latent fingerprints on metallic surfaces, and will learn about latent fingerprint residue components, how these are affected by exposure to different elements, and about nontraditional development techniques for latent fingerprint development on metallic evidence.

This presentation will impact the forensic science community by sharing important information that can lead to the successful development of more identifiable latent fingerprints on a variety of metallic surfaces.

Traditional latent fingerprint development techniques, such as cyanoacrylate fuming and powdering, have relatively low success rates on metallic surfaces on forensic evidence, mostly due to the nonporous nature of the metals, but also due to the fragile nature of the latent fingerprints. A latent fingerprint is composed mostly of bodily secretions (e.g., water, inorganic salts, amino acids, fatty acids, ammonia, and urea), which can degrade under extreme environmental conditions, such as high temperature and low humidity. The survivability of a latent fingerprint on any surface (metal surfaces included) depends on many factors including atmospheric conditions (e.g., temperature and humidity) and contaminants (e.g., dust, water, mud, and surface coatings). Traditional latent fingerprint development techniques rely on the physical and/or chemical interaction of the "developer" with the unaltered components (i.e., specific functional groups) of the latent fingerprint secretions. Therefore, traditional techniques are not very effective when the compounds in latent fingerprint secretions have been altered or degraded, as occurs when evidence is aged or has been exposed to extreme environmental conditions. Metals are nonporous in nature and, therefore, do not absorb fingerprint residues, rendering fingerprints exposed and, hence, easier to degrade and/or obliterate under extreme environmental conditions and/or when in contact with contaminants. Moreover, metals are excellent heat conductors; therefore, a metal surface will tend to heat faster and more uniformly than other surfaces, potentially exacerbating the degradation of the latent print residues.

Metallic evidence is critical in the forensic arena given that many weapons are constructed with metallic components. Many weapons are exposed to high temperatures, low ambient humidity, rough surroundings, and a variety of surface contaminants. Firearm cartridge casings, for example, undergo surface deformation and heat exposure when they are fired. Also, many weapons have anti-corrosive coatings or oily residues present on their surfaces, making traditional development techniques quite ineffective.

A variety of nontraditional latent fingerprint visualization techniques for metallic surfaces (e.g., modified small particle reagents, gun bluing, corrosion/oxidation etching, and nanoparticles) have been studied and modified accordingly. Natural and synthetic (sebaceous and amino acid) fingerprint residues have been placed on metallic coupons and have been subjected to controlled extreme environmental conditions (i.e., high temperatures and low relative humidity) using an environmental chamber in an attempt to degrade latent fingerprint residues. Degraded prints have been treated with traditional and nontraditional development methodologies for comparison, and developed prints have been assessed for quality and quantity of detail. The new methodologies are being evaluated to improve latent fingerprint recovery from metallic evidence, either as a replacement for or in combination with traditional nonporous processing protocols.

Latent Print, Development, Metal Evidence

D75 Evaluation and Selection of Touch DNA Evidence Using Decision-Making Software

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After attending this presentation, attendees will gain understanding about how touch DNA evidence is evaluated and selected using decision-making software for DNA analysis based on an effective case strategy. Case examples will be presented which explain how the approach is used in selection and analysis of different types of touch DNA evidence.

This presentation will impact the forensic science community by providing a business method to manage selecting suitable evidences for touch DNA analysis. This presentation will enable crime scene, forensic laboratory, criminal investigation, and prosecution to make the most of touch DNA evidence.

Touch evidence DNA is the widely used invisible evidence to prove the possible presence of biological material on a surface to connect a person to the crime scene. As the suspected surfaces for touch evidence could be large and numerous, this will increase analysis time, increase case backlogs, and increase the cost of the analysis which are considered to be major challenges to the forensic laboratories in the era of the current international financial crisis. In order to solve this problem, decision-making software was used to reduce the number of touch evidence cases, reducing the cost of analysis, and producing meaningful results to assist in the case investigation. The method will start by applying an effective case strategy management procedure, followed by the use of a decision-making program in order to evaluate different evidence according to certain criteria. An effective case strategy management includes identifying case requirements and needs through the main stakeholders. The requirements in each case can be categorized into two main sections, which are the identification

processes of the victim or the suspect and crime scene reconstruction. The case stakeholders were identified to include all main persons, groups, or organizations which are related to the victim(s), the suspect(s), criminal investigation department, prosecution, and court. After understanding the requirement of the case, touch DNA evidence will be evaluated and selected using decision making software. The V.I.S.A® (Visual Interactive Sensitivity Analysis for multi-criteria decision-making) program was used as a decision-making tool to select the most useful touch DNA evidence for the case. Possible touch DNA evidence such as shoes, pens, cans, watches, seats, cigarette, and clothes will be evaluated in the software according to differential criteria such as contamination level, DNA damage condition, level of information gained, connection to victims, connection to suspect, connection to witnesses and location in the scene. Each criteria has its own sub criteria such as contamination level which can be low or high or without contamination. A decision tree model will be constructed for the evaluated evidence. This type of filtration process can be done in the laboratory after collecting the necessary evidences from the scene. In a case example, a body was found outside a labor camp, the deceased had a blunt force wound to his face, was fully clothed, and his mobile phone was found near him. Two people were arrested in relation to the case. Initially both denied being involved in the case. Following discussion with the main stockholder of a criminal investigation department, the identification of the suspect was considered the priority. A large number of items were received for examination. Few touch DNA possible evidence were selected and several similar evidences types were excluded from analysis after evaluation on the above criteria. The selected samples in the case were enough to connect the suspect to the victim. The approach was useful in this case to reduce the cost and time in selection touch DNA evidence. It is recommended that the forensic scientist, investigator, and prosecution apply an effective case strategy management, in addition to the use of decision-making software to select and evaluate large numbers of different touch DNA evidences. These methods also can be used in other types of forensic samples. More research is needed in the field of using business management techniques in the evaluation and selection of forensic evidence.

Touch DNA, Selection, Decision-Making

D76 Accuracy of Latent Print Identification Using AFIS

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The goals of this presentation are to simplify the comparison process, determine a threshold for the adequate amount of minutiae points needed for an identification and determine the error rates that are made by latent print examiners.

This presentation will impact the forensic science community by providing necessary data for laboratories to evaluate the advantages and disadvantages of using a numerical standard.

Minutiae have been used in the past as a measure for effecting identifications of latent fingerprints. In 1973, the International Association for Identification issued a statement regarding using minutiae for identifying latent fingerprints. The consensus was that there is no basis for using minutiae as a guideline to make identifications. Since then, there have been fluctuating opinions regarding its use. The purpose of this study will be to define error rates in latent fingerprint identification using receiver operating characteristic (ROC) curves. The study proposes to simplify the comparison process by making two assumptions: firstly, only minutiae will be used in the identification process; and, secondly, only specific, clearly identifiable ridge endings and bifurcations will be used. In the baseline study, latents of varying size (and thus varying volumes of identifying features) will be used to gain estimates of error rates.

Preliminary studies, comparing latents with varying numbers of identifiable minutiae using an AFIS, have provided interesting results. Examiners were given a number of latents and a variety of known prints. The prints were then entered into an AFIS. Three specific areas of the latent prints were focused on: tip, core, and delta. For each print, a separate analysis was performed at each of the specified areas. The analysis included selecting from 3 – 50 identifiable minutiae around the designated areas. The frequencies of minutiae combinations were recorded by choosing from 3 – 5 minutiae in relative closeness to the area of concern. The results can be analyzed in a number of ways using signal detection theory and compared for model predictability. The ROC curves will also provide a measure of discrimination irrespective of the method which the examiner provided.

In the baseline study, the error rates were defined in latent fingerprint identification using AFIS. The effectiveness of identification using AFIS was evaluated using receiver operating characteristic curves. The prime objective was to be able to clearly define how the number of minutiae can be used to understand and be applied to error rates. All minutiae are not valued to the same degree, highlighting the need for different numerical standards for the three areas that have been chosen for analysis. These standards will be used to determine the threshold and how it changes based on how the latent occurred. One must also consider at what point this threshold will change based on the area (tip, core, or delta) chosen for analysis. Clear definitions of false positive and false negative rates and their implications for latent fingerprint examiners will be established.

The results of this study may provide the necessary data for laboratories to evaluate the advantages and disadvantages of using a numerical standard. According to the Inspector General's report into the Brandon Mayfield Case, a numerical standard is where a minimum number of points must be in adequate agreement for an identification.[1] The forensic community could benefit from this adoption because "the premise of establishing such a standard is that the probability of encountering two different fingers that share that number of minutiae in common is infinitesimal and can be disregarded" which was also stated in the Inspector General's report.[1]

Reference:

1. "A Review of the FBI's Handling of the Brandon Mayfield Case," Office of Inspector General, U.S. Department of Justice, March 2006, www.usdoj.gov/oig/special/s0601/final.pdf.

Latent, AFIS, ROC Curves

D77 Working Together: Utilization of Outside Resources When Dealing With a Mass Fatality

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After attending this presentation, attendees will learn the importance of resource utilization by the Medical Examiner's Office when dealing with a mass fatality.

This presentation will impact the forensic science community by detailing how a small Medical Examiner's Office responded to a mass fatality incident on a major interstate, effectively, by identifying available resources and calling upon them at the onset of an incident. Instead of waiting until the scene of a mass fatality has been processed, or getting to a scene and realizing more help is needed, resources were called upon at the onset of the incident, thus allowing for an effective and precise investigation.

On January 29, 2012, fog and smoke from nearby wildfires blanketed a low-lying area of Interstate 75 in Gainesville, Florida, at approximately 0400 hours. Vehicles traveling on the roadway at high speeds suddenly experienced zero visibility, resulting in a multi-vehicle crash on the northbound and southbound lanes of the Interstate stretching a little over one mile. The Medical Examiner's Office was notified that multiple fatalities were confirmed on scene at approximately 0600 hours.

The Medical Examiner's Office consists of three full-time investigators, one part-time investigator, two autopsy technicians, and three pathologists. When the on-call investigator was notified of the

incident, contact was immediately made with the Chief Medical Examiner and the Director of Operations. Quickly, it was established that the Florida Emergency Mortuary Operations Response System (FEMORS) should be activated and deployed to offer resources and staff. All team members, which included all Medical Examiner's Office staff, FEMORS personnel, and transport personnel met at the Medical Examiner's Office at 0800 hours for a briefing.

FEMORS personnel started a Victim Identification Center (VIC) that allowed for people to report missing individuals that may have been involved in the crash, providing resources for identification. At the time of the crash, there were eight unidentified individuals. Because the VIC was implemented at the onset of the incident, antemortem information was readily available to guide the process of gathering postmortem comparison information.

The northbound lanes consisted of multiple vehicles with deceased located in two separate vehicles. Two decedents were in a small passenger car and five decedents were located in a minivan. With the assistance of FEMORS personnel, scene photography, scene sketching and decedent photography was accomplished efficiently in various stages. Prompt processing of the northbound scene allowed for decedents to be transported to the Medical Examiner's Office for processing and autopsy. FEMORS personnel located at the Medical Examiner's Office were instrumental in being able to process the decedents as they arrived to assist the two autopsy technicians and pathologists.

The southbound lane crash involved two vehicles that had become fully engulfed in flames from two semi-tractor trailers catching on fire. The result was that two vehicles with decedents were severely burned. At this time, the Medical Examiner's Office contacted the C.A. Pound Human Identification Laboratory and a board certified forensic anthropologist responded to the scene. This allowed for an expert opinion on the most effective way to preserve the burned remains. The majority of the remains of one individual from one vehicle could be removed on scene. An unknown number of individuals remained in the second vehicle. On the advice of the physical anthropologist, both vehicles were towed to a local law enforcement facility for later processing.

From January 30, 2012, through February 2, 2012, both vehicles were processed by a team of physical anthropology graduate analysts, as well as forensic odontologists from FEMORS. The expert archeological techniques and knowledge of burned bone identification and preservation allowed for maximum recovery of remains as well as identification of personal effects. Having forensic odontologists at the site allowed for *in situ* examination of dentition given the extremely fragile nature of the remains. In all, three individuals were recovered from the second vehicle.

The proactive measures of the on-call investigator and the Medical Examiner's Office team to identify, call upon, and utilize outside resources to their fullest extent allowed the Medical Examiner's Office to effectively identify the remains and investigate the deaths of eleven individuals.

Mass Fatality, Investigation, I-75

D78 Homicide vs. Suicide: Suicide by Cop Revisited

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After attending this presentation, attendees will understand the principles of suicide vs. homicide investigation and the application of an operational methodology to the investigative process to assist in the determination of the classification of manner of death.

This presentation will impact the forensic community by demonstrating the use of an operational methodology to guide and assist the standardization of the investigative process when investigating suicides, especially when the suicide involves subject-precipitated homicide.

Suicide by Cop (SbC) is a well-documented phenomenon in the forensic literature. Demographics as well as motivational considerations for SbC have been researched and detailed. But less clear is the operational methodology which investigators, including medical examiners and coroners, use to classify these incidents. Among investigators, there appears to be less reliance on standardized operational criteria for SbC and more reliance on outside, often emotional, influences such as family and social stigma, equivocal deaths and variability among medical examiners and coroner systems. As a result, these incidents may be misclassified, leading to questionable epidemiological data with regard to the problem of suicide nationwide. Homicides and suicides in the United States, and especially among members of the military, are of great concern to public health officials. Public health policy is based, in large part, on these statistics. Statistical error can result in misapplication of programming decisions with regard to reducing incidents of homicide and suicide, leading to much wasted time, effort and program dollars.

Operational criteria have been established in the literature: "*Operational Criteria for the Determination of Suicide*" (OCDS) was published in which criteria were set forth for determination of suicide;[1] "*Empirical Criteria for the Determination of Suicide Manner of Death (EDOS),*" was developed in which criteria were set forth for determination of suicide;[2] "*Police Shooting as a Method of Self-Harming: A Review of the Evidence for "Suicide by Cop" in England and Wales Between 1998 and 2001,*" developed methodology for determination of SbC based on observable acts;[3] and, "*Suicide by Cop: Police Shooting as a Method of Self-Harming,*" worked out a set of indicators to help classify SbC.[4]

A case study will be presented in which a 64-year-old white male with a history of suicide threats and addiction to drugs and alcohol, called his daughter threatening suicide by shooting himself. Law enforcement was notified and the male was subsequently shot and killed by a responding sheriff's deputy after the male pointed a shotgun at him to shoot the deputy. The shooting was ruled a homicide. The case will be analyzed using standards presented in the operational criteria discussed in the literature to determine if the case should have been ruled more properly as a suicide. A recommendation will be made that medical examiner systems and coroner systems such as that in Ohio adopt an operational methodology for the determination of suicide and in particular SbC.

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Suicide, Suicide by Cop, Public Health Policy

D79 Case Study: From Maternal Instinct to Staged Domestic Homicide

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After this presentation, attendees will understand how, through a process of comprehensive analysis of the crime scene, was obtained a reconstruction of a criminal behavior and which was the driving motivation of a murder against two children perpetrated by their own mother.

This presentation will impact the forensic science community by showing how the criminal conduct of staged domestic homicide has some repetitive patterns or common characteristics in terms of planning and execution, regardless of where in the world presented, without being influenced by sociocultural or economic factors of their executors.

One mother told the police of the city of Mocoa, capital of the department of Putumayo in southern Colombia, in the early hours of September 20, 2010, an unknown individual attacked when she opened the front door of her residence, and she was attacked until she lost consciousness. After regaining consciousness, she observed that their children were in bed in the master bedroom and, when approaching them, believing them to be asleep, noted they were wet and without signs of life.

At the beginning of the investigation findings in the scene, the woman´s injuries were consistent with blunt trauma and the apparent coherence of her story led the authorities to think that there was a perpetrator who murdered two children and had left their mother seriously injured. The high social impact produced by a case of two children killed and the assault on their mother activated law enforcement, both local and national level, so that the Criminal Behavior Special Unit of the Attorney General's Office was called to support the investigation through a process of crime scene analysis and the possibly developing a criminal profile.

On the basis of the processes of crime scene analysis, 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 crime being committed by the mother.

This change in the case offered to 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 of finding the motivation that drove this woman to kill her own children. Therefore, the line of investigation focused on probing deeper into all areas of the life of the mother, and found an intricate world of love frustrations and abandonment, a mentally unhealthy woman, egocentric and possessive, with strong intentions to pass over any obstacle to achieve all personal desires.

After harmonizing and 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 mother, in the form of staged domestic homicide. Today this woman is listed as a fugitive from Colombian justice.

Mother's Killer, Children, Staged Domestic Homicide

D80 The Patricia Flores Cold Case: An Interdisciplinary Approach About Death and Rape After an Investigation in a School in La Paz, Bolivia

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After attending this presentation, attendees will get a well-rounded view of a forensic cold case investigation in Latin America by showing the interdisciplinary forensic work about the murder and rape of a 9-year-old girl in 1999 in La Paz, Bolivia.

This presentation will impact the forensic science community by explaining the importance of the interdisciplinary work, consisting of pathology, archaeology, anthropology, psychology, and crime scene investigation, in order to investigate cold cases related to child homicide and abuse in Latin America.

On Friday August 27, 1999, Patricia Jaqueline Flores-Velasquez, a 9-year-old girl living in the city of La Paz, Bolivia (South America), disappeared from the public school she was attending and four days later her body was found inside the school warehouse. The body showed signs of asphyxia, rape, and torture. Once the body of the girl was discovered at 11 p.m., there were several mistakes related with the crime scene investigation, including analysis and technical/scientific procedures, chain of custody, the absence of sexual abuse protocols, and a proper necropsy. The Medical Examiner committed suicide just few days after Patricia's body analysis was done, apparently because of bad practices used in this case.

DNA samples were taken by the FBI of two suspects. In one of them, Patricia's DNA was found in a belt and in a shoe of the suspect, but the Bolivian court did not accept this evidence and argued contamination. The case became a cold case.

After 11 years, the criminal process was re-opened thanks to two Human Rights NGOS named Funderes (a Human Rights non-profit organization from Bolivia) and Women's link, (an international human rights non-profit organization working to ensure that gender equality is a reality around the world).

In April 2011, a group of 11 forensic scientists and criminal investigation analysts (seven Colombians and two Spaniards) started to work the case, analyzing documents, testimonies, and expert witness reports in order to help the Bolivian Justice to again open the case with forensic evidence

In August 2012, an interdisciplinary team composed a group of Colombian forensic scientists and technicians (one anthropologist, one pathologist, one crime scene analyst, one forensic photographer) who belong to the National Institute of Legal Medicine and Forensic Sciences and AFFIC foundation, went to Bolivia in order to do an exhumation of the body, a second necropsy, took new DNA and other biological samples from the body, suspects and forensic evidence, and conducted forensic psychological tests on suspects and a new analysis at the crime scene.

A group of forensic psychologists from Colombia and Spain worked on a psychological profiling in this case, including the suspect's behavior, motives, and background, in an attempt to guide the investigation. Psychologists gave a profile using both inductive and deductive approaches, trying to find if the murder was committed by a serial killer or was an isolated case.

Cold Case Bolivia, Child Abuse, Sexual Abuse

D81 Homicide or Suicide: The Shotgun is Twenty-One Feet, Six Inches From the Victim's Body

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After attending this presentation, attendees will understand the necessity of experimentation to prove or disprove a theory when it involves criminal investigations. Students will receive information on a specific case where a 12-gauge pistol-grip pump shotgun was found approximately 21.5 feet away from the truck where a victim was found in the driver's seat with a contact gunshot wound to the chest. Determining how the shotgun got that far from the vehicle where the deceased was found was one piece of the puzzle to help to determine homicide or suicide.

This presentation will impact the forensic science community by showing how experimentation to prove or disprove, teamwork of forensic disciplines, and good old-fashioned investigation can come together to provide a more confident conclusion in determining the outcome of an equivocal investigation.

On January 10, 2011, a white male entered a local market and purchased a random pick lottery ticket for a date in the future. A few minutes after leaving the store, the store owner and his adult son heard what sounded like a backfire or a gunshot. The adult son went outside to the parking lot and found a white Ford F-350 pickup truck with the driver's window down and the white male lying back against the driver's seat, bleeding from the chest.

When emergency personnel arrived, it was evident the white male was deceased. The scene was secured by local law enforcement. Detectives noted a Mossberg 12-gauge pistol grip pump shotgun 21.5 feet away from the driver's door of the truck. In addition, there was not any visible blood found on the shotgun. The driver's side mirror was spattered with blood and a few drops of blood were found on the ground just below the mirror. The ignition was in the "on" position with the radio on, but the truck was not running.

The manner of death is partly based on investigation, forensic evidence, and sometimes good old-fashioned gut feeling or experience. Facts, forensic and investigative, are the only parts of information that really count when it comes to making a decision on what happened. In this particular investigation, theories were developed as to how the shotgun ended up 21.5 feet from the victim's body. Gut feelings are not facts, but are merely a platform to develop a theory or theories. Experience is not a fact either it is just a better way to develop a more accurate prediction of "what happens when." When experiments are conducted, "what happens when" can often end up with a surprising result, either proving or disproving a theory.

When a theory is developed, it needs to be proven or disproven using scientific method. As much as possible, the original circumstances need to be replicated. In this particular investigation, the original shotgun and same type of ammunition were used. All other circumstances had to be replicated by using a different truck, but in a location with a similar type of asphalt surface. Cushions and clothing were used to construct a dummy in which to shoot. For safety purposes, the experiments were conducted at a police range under the supervision of the police range master.

The key to experimentation is planning and flexibility. Accounting for every circumstance is impossible; therefore, adjustments in methodology and/or the hypothesis may have to be considered. Flexibility is important to be able to make the adjustments during experimentation. Keep in mind things can happen more than one way. In this case, more than one person was surprised with "what happens when."

Theory, Scientific Method, Experimentation

D82 The Importance of Autopsy in Deaths Due to Choking

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After attending this presentation, attendees will learn about the importance of autopsies in cases with suspected deaths due to asphyxia from choking on foreign bodies which, even when material has not been identified by emergency medical responders and caregivers, warrant an autopsy.

The presentation will impact the forensic science community by emphasizing the importance of performing an autopsy on choking-related deaths, which may have medicolegal repercussions.

In the United States, choking and suffocation accounts for the third leading cause of home and community deaths, with young children and older adults at the highest risk.[1] The lack of adequate investigation, including a formal autopsy performed by a forensic pathologist, may result in the underreporting of asphyxia deaths. In the forensic community, asphyxia from choking is defined as an obstruction from food material or foreign objects within the airways below the epiglottis.[2,3] The manner of death is most commonly an accident. Besides age being a risk factor in these cases, neurologic or mental deficits, poor dentition, and alcohol intoxication are among the most common underlying reasons that increase the likelihood of choking-related asphyxia deaths.[2]

In medical forensic cases where a suspected asphyxia from choking on food occurs, emergency medical responders and caregivers will often recognize the signs of an airway obstruction and attempt to remove the food item from the airway. However, during a review of the Wayne County and Washtenaw County Medical Examiner database from 2008 to 2012, 20 cases in which food products were identified below the epiglottis at the autopsy table had not been previously identified by emergency medical responders and caregivers at the accident scene.

Information obtained from a review of the identified cases included age, predisposing risk factors, significant neurologic or mental comorbidities, on-site findings by emergency medical responders and caregivers, and final autopsy findings. In this review, decedents ranged from the age of 13 months to 89 years old, many had risk factors that included missing teeth and acute alcohol intoxication, and several carried a previously diagnosed comorbidity, including mental retardation and dementia.

Initial on-site medical response efforts in these choking cases failed to properly identify remaining food material in the airways, most likely due to the location of the food material being lodged below the epiglottis and away from direct visualization. At the autopsy table, proper visualization of food material below the epiglottis, which obstructed the decedent's airways that lead to eventual death, could more easily be identified in all cases. These autopsy findings, given the circumstances, were assigned a cause of death due to asphyxia by choking, which was accidental in manner. This highlights the importance of performing an autopsy in suspected choking-related deaths because the cause of death may be otherwise overlooked and potential medicolegal repercussions are at stake.

As noted, a significant portion of the cases in this review involved decedents with previously diagnosed comorbidities and risk factors. Initially, some forensic pathologists may choose to assign a natural cause of death in these cases based on the on-site response finding of no food material combined with a history of a neurologic or mental deficit and/or predisposing risk factors, including acute alcohol intoxication. This may lead to the decision to either forego a formal autopsy or perform a limited external exam with toxicology studies. However, these medical forensic cases warrant a formal autopsy due to the possibility of significant findings at the autopsy table, including complete obstruction of the decedent's airways below the epiglottis.

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Autopsy, Choking, Asphyxia

D83 Suicide in Children: A Study in Northern Portugal

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After attending this presentation, attendees will understand information related to suicidal behavior in children and adolescents and make them aware about the need for more education, specifically by promoting a thorough psychological autopsy in these cases.

This presentation will impact the forensic science community by shedding light on a poorly studied area of forensics in northern Portugal, the iddue of child and adolescent suicide, emphasizing the requirement to develop understanding in managing autopsy cases to be supported by specific strategies.

Suicide is among the leading causes of death worldwide and is the second leading cause of death between 10 and 24 years old. Suicide behavior includes a wide spectrum of acts, with males tending to choose methods with more lethal effectiveness, making them more likely to perform a complete suicide. Lack of information about some circumstances of the death, namely underlying psychopathologic disorders and the behavioral impulse, contribute to the suicidal act; this can create flaws concerning the method of the psychological autopsy. Portuguese forensic aspects on child and adolescent suicides are unknown, so that was the aim of the present study.

Data on autopsy reports of suicide cases of people under 18-years-old at the North Services of the National Institute of Legal Medicine and Forensic Sciences of Portugal, from 2004 to 2011, including toxicological results, were obtained, and psychological autopsy data and police reports were analyzed. A total of 16 cases were found, with a male predominance (68.8%) and an age range from 12 to 17-years-old (mean age 15.25 ± 1.13). All victims but two were Caucasian. The leading suicide method was hanging (n=6), followed by shooting (n=3), and jumping in front of an oncoming train (n=3). A greater number of suicides (n=9) took place between June and September. Seven cases occurred in public areas and six at the victim's home. Alcohol intoxication was observed in two victims (blood alcohol concentration > 1.0g/L), both males, who committed suicide by jumping in front of an oncoming train. Psychological autopsy was performed in only 10 cases, which can be explained by the difficulties of forensic professionals leading during a quite early stage of grief, and also by the fact that family and friends of the victims are having trouble with providing accurate information in that moment, since they aren't able to cope with the pain, which affects and impairs their memory and concentration. This suffering can lead to feelings of blame, associated with inaptitude of accepting the suicide event. Therefore, it's highly important to develop specific competencies for the forensic professionals for this type of approach, namely in early stages of the grief process. These are some of the aspects which could explain why the main reason for suicide was not possible to determine in most cases.

Although suicide in children and adolescents is a rare event, it remains a rather important issue of public health, and should not be overlooked. Such events are probably motivated by acts of impulsiveness, thus these acts are usually unpredictable and come as a surprise to the family and friends of the victims. The chosen methods tend to be more violent than in other age groups, namely in males, which explains the gender predominance. It is of paramount importance to assure the correct course of action regarding the psychological autopsy in these cases, as well as the availability of information regarding the victims, including reviewing of the clinical history, social and emotional background, hence creating a more complete profile of these victims and maximizing the accuracy of the forensic diagnosis. This is the first study of suicide in victims under 18 years of age in the north of Portugal, and offers unique information for future programs of suicide prevention in this country and a basis for further and needed research. Prevention strategies should focus on controlling the main risk factors, namely awareness of underlying psychiatric pathologies, acts of impulsive aggression, family history of suicide, history of physical or sexual abuse, or poor relationships with parents and peers, as well as restricting the access to lethal methods, therefore promoting early assessments leading to targeted interventions and reducing the risk of suicidal behavior.

Suicide, Children, Risk Factor

D84 Epidemiology and the Cost of Falls: Monitoring the Last 22 Months at the Policlinico Hospital of Bari

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The goal of this presentation is to show the results of a retrospective study on falls occurring at the Policlinico Hospital of Bari, Italy, in the period March 1, 2010 – December 31, 2011. Patient data and fall incident report data have been reviewed to identify risk factors associated with falls and fall-related injuries. Moreover, a systematic approach led to estimates of the average hospital cost and Length Of Stay (LOS) associated with injurious falls in the hospital.

This presentation will impact the forensic science community by offering useful insights to the forensic science community by demonstrating how important risk management can be, also, in order to save money that could be better spent for healthcare services, whilst on the other hand, it highlights how forensic sciences, especially in recent years, have focused attention on patient health and safety.

Indeed, the growth of healthcare costs over the last years has fostered careful scrutiny of both the effectiveness and efficiency of healthcare delivery.

According to this, the main purpose of the Clinical Risk Management System is to ensure patient safety through the implementation of systemic measures of identification, analysis, evaluation, and treatment of risks related to the provision of care.

Such an ambitious program aims to reduce the probability of incidence of an adverse event/error or, at least, to limit the impact of the consequences of such an event also from a "hospital efficiency" perspective.

Falls, which are not strictly related to the care process and in consideration of their possible consequences, usually entirely separate from the cause of hospital admission, represent a critical organizational node. In fact, according to the literature reviewed, they (falls) are among the most frequent adverse events—especially in acute care facilities—and they can result in substantial morbidity and mortality. Particularly, falls may lead to negative outcomes such as injury, prolonged hospitalization, delayed rehabilitation, and potential professional liability profiles.

Rates between 2.2 and 17.1 falls per 1,000 patient days, depending on hospital type and patient populations, have been reported.

The correlation between potential frequency and severity of falls identifies the key role played by them in the risk map. At the same time, it is well known that falls mainly occur in the elderly, especially amongst people suffering from neuro-psychiatric disorders, and generally related to voiding and/or toileting requirements.

The potential risk factors of falls have been studied in depth and, consequently, various hospital falls-prevention programs have been implemented in the last decades. However, most of the programs had no

sustained effects on fall reduction over extended periods of time. In this sense, it is certainly time for the forensic community to monitor the phenomenon and to play an active role in the implementation of corrective measures.

With this in mind, at the Policlinico Hospital of Bari a surveillance system based on the distribution and filing of "Fall of the Patient" record cards has long been in place.

Even though it is unrealistic to consider all falls to be preventable, it is hypothesized that a system-based fall-prevention program targeting high-risk situations would result in fewer falls. According to this, hospital managers have a leading role in creating an effective program and reducing hospital costs.

Falls, Risk Management, Epidemiology and Cost

D85 Differentiating Perpetrator from Witness Using BEOS Profiling

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After attending this presentation, attendees will learn about a new technique for forensic investigation and deception detection.

This presentation will impact the forensic science community by advancing research that is strongly needed in new areas of crime investigation.

Brain Electrical Oscillation Signature (BEOS) Profiling is a technique used to identify individuals with remembrance of specific experiences. The test detects the presence of specific patterns in the electrical signals (EEG) recorded from the brain, while listening to verbal statements used for cueing remembrance of components of a specific experience, acquired while committing a crime or forensically relevant act. Remembrance of autobiographic episodes may have components of motor and visual imageries associated with the actions one has executed and witnessed. If one has only witnessed a scene, he cannot have motor imageries of carrying out the same act, but will have only visual imagery. Remembrance of components of autobiographic episodes contributes to greater neural activation compared to retrieval involved in knowing. Remembrance is found to be often associated with recreation of motor and visual imageries. In BEOS Profiling, short narrative sentences are presented in sequence referring to several of the activities a suspect or accused is considered to have indulged in. The procedure can be used to test an investigator's as well as a suspect's version of the involvement of the latter.

The current study was conducted in the BEOS Laboratory at the Directorate of Forensic Science Laboratory, Mumbai, India. A participant in the experimental group executed an activity according to a pre-designed action plan, when he was accompanied by another participant from the witness group. The later merely witnessed the actions executed by the former. The activities in the experiment room consisted of playing a numerical game, searching for keys, a doll, tools, boxes, and opening the box, breaking a large flowerpot, collecting coins from it, and finally leaving the room. There were 20 pairs of participants and 13 participants in a control group. Subsequently, the BEOS test was carried out after an interval of 15 days on each participant of the experimental group. A control group of participants who did not take part in the above experiment were also administered the same probes while acquiring their EEG. The probes referred to the activities of the participant who carried out the activities in the experimental room. Several of them required the participant to recreate either both motor and visual imageries or one of them. The participant sat silently listening to the probes with his eyes closed and without giving any response.

The maximum length of a probe was never more than three sec. The EEG was acquired in the range of 0.016 – 100 Hz by Neuro Signature System, using 30 channels of scalp electrodes. Vertical and horizontal eye movements, if present, were recorded in another two channels. Continuous EEG was acquired and it was converted to 10 sec epochs, time locked to the onset of each probe, with three sec pre-probe baseline and seven sec of response segment. Analysis consisted of frequency and time domain analyses followed by statistical comparisons of the patterns in the response segment with the pre-probe baseline values. Frequency analysis was carried out in 10 frequency ranges and the time domain analysis consisted of detection of positive and negative responses of predefined morphology in comparison with the baseline levels. Significant increases in the Coherence values across pairs of fronto-central and fronto-posterior electrodes in the 35 – 85 Hz range in the response segment compared to the baseline indicated the presence of motor and visual imageries respectively.

Specific pattern of significant changes in the power spectrum values in the response segment, presence of significant increase in the phase relationship in gamma range of activities, and presence of significant ERP components indicated the presence of remembrance, which was marked as Experiential Knowledge response. The analysis indicated the presence of motor or visual imageries in response to each probe, if present. The number of probes, which produced such changes, was identified in each participant, and the scores were compared across the three groups. Comparison of results of motor and visual imageries across groups indicated significant increase in motor imageries in the perpetrator group whereas visual imageries were comparable in the perpetrator and witness groups. The control groups had significantly lesser number of EK responses.

Perpetrator, Witness, Motor and Visual Image

D86 The Phenomenon of the Urban Mummy

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After attending this presentation, attendees will have an understanding of the constellation of environmental factors and events leading to human mummification in urban settings, and the importance of the multidisciplinary approach to the medicolegal investigation of such deaths.

This presentation will impact the forensic science community by contributing to the increased awareness of the study of these unusual cases, and the unique aspects of the medicolegal investigation of these deaths.

The environmental mummification of a body in an urban setting is a fairly unusual event. Florida is known for its high temperatures and humidity, which typically contribute to the rapid decomposition and putrefaction of decedent's bodies. The resulting foul order is frequently the signal leading to the discovery of the bodies of individuals whose deaths occurred in solitary locations. This, coupled with prevalence of insect activity, usually precludes the mummification process. However, under certain circumstances, Florida's unique climate provides an environment suitable for mummification. A particular constellation of human behaviors and scenarios must coincide for this phenomenon to occur in an urban setting.

The first requirement is that the decedents must have lived in socially isolated circumstances. Such isolation may result from multiple etiologies, such as illness, substance abuse, criminal activity, language barriers, and/or advanced age. Florida is home to a large number of individuals who lead an isolated existence: the homeless, who may purposely isolate themselves as a safety measure to protect themselves and their property; the mentally or physically ill, who are isolated as a result of their impairments; and, the elderly, whose longevity may result in their isolation after the deaths of friends and family, are all at risk. Reclusive behavior is almost essential for a death to go unnoticed and to meet the time requirements for the decompositional mummification process to proceed.

The apathy of nearby individuals to the welfare of the decedent or the lack of knowledge of the decedent's presence in the community is another factor. If neighbors call attention to the absence of the decedent, a well being check will usually interrupt this process. The failure of public officials to recognize the signs of a potentially

undiscovered death is also a necessary condition. These may include law enforcement, mail carriers, and/or public safety officials. Even with isolationist behavior, violations of urban laws or ordinances may result in the inspection of a home by public officials. These public service representatives must be apathetic to the absence of the decedent or equate his or her absence with the abandonment of the dwelling. Finally, the body must be in a hot, dry, closed environment where it is protected from insect and animal predation. The death of an individual in an exposed location is often appreciated by the local fauna as a ready food source, long before humans become aware of the death. This predation results in the consumption and scattering of the remains rather than their mummification.

Several cases in which mummified bodies were discovered in urban locations in southwest Florida have recently been investigated. The literature pertaining to such unusual cases is sparse. These cases highlight the importance of the multidisciplinary approach to the medicolegal investigation of such deaths and the determination of the cause and manner of death.

Urban Mummy, Medicolegal, Investigation

D87 Examinations of the Standards of Normality (SON) and Production Standards (PS) to Best Identify Critical and Definitive Information Pertaining to Alleged Nutritional Associated Dysfunctions (NAD) in Animals

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After attending this presentation, attendees will understand how to carefully reconstruct an animal facilities Standards Of Normality (SON) and Productions Standards (PS) critical to identifying their relationship to animal death and abnormalities attributed to primarily a Nutritional Associated Dysfunction (NAD).

This presentation will impact the forensic science community by providing the key components necessary to properly investigate alleged animal nutrition-related deaths or abnormalities attributable to daily rations provided to commercially raised animals.

When the use of animal feeds in commercial livestock facilities result in abnormal PS, mortality rates and/or lower-than-expected performance and health parameters, it is important that a comprehensive animal and SON diagnosis be performed

Today's production livestock are finely tuned "food producing machines" that require a balance of nutrition, environment, health care, and management; any deficiencies or inadequacies in these areas will affect the ability of an animal to perform optimally. When investigating a manifested dysfunction in a livestock operation, proper analysis for each area—nutrition, environment, health care, and management—insures that the evaluation is as scientifically accurate and valid as the data permits.

An essential component in the evaluation of animal feed-related claims is a scientifically valid set of investigative techniques required to accurately evaluate Feed Associated Dysfunctions (FAD) and NAD in animals. As an example of the practical application of these techniques, the author will present a case study involving a large dairy and a toll manufactured nutrition product alleged to be inadequate. The testing and identification of milk fever, immune system abnormalities, death, and damages to the cows' reproductive system and milk production, will be used as the focal point in providing insight into measuring and documenting causation for the professional investigating similar situations.

Formulating dairy rations primarily involves providing specific fibers, protein, energy, vitamins, and mineral ingredients for defined maintenance, reproductive, and productions requirements. Several factors affect a cow's requirement for a specific nutrient. These factors influence feed intake, which will require changing the concentration of the nutrient in the diet to meet the cow's requirement on an amount-per-day basis. This presentation will identify common factors which influence specific nutrient needs and their importance to a comprehensive NAD investigation.

The physical condition of the animal's habitat and associated living organisms that occupy it must be considered in all forensic cases involving animals. The dynamic whole of the microenvironment that immediately influences the target animal individual or population exists as an interdependence of its members.

In microenvironments such as a dairy barn, or outdoor feeding area, the targeted individuals or populations that occupy it and their nonliving environment are inseparably related and are constantly interacting with each other. The exchange of materials between the living and non-living parts of a target animal or population are intimately involved in the determination of causation in nutrition-related animal death, illness, or abnormalities.

Modern animal nutrition forensic investigations cannot separate its observations from the complexity of the environmental realities that act singly and together. At the same time, it is recognized that the animal or targeted population in turn react upon their environment, often producing marked modifications.

Problems that occur in groups of animals are often multi-factorial in cause and the result of the interaction of several risk factors, which may be characteristic of the animals, their environment, their nutrition, and/or inciting agent. In the context of an operation such as a dairy facility, managerial causes must be analyzed and either ruled out as a causative factor or incorporated and weighted as a factor accordingly.

In order for Nutrition Associated Dysfunctions (NAD) to be properly investigated, documented, and analyzed to determine causation, reconstructing daily diets, SON, and PS is essential. This presentation will provide the basic scientific techniques needed to gather evidence to determine if feed has been formulated with sufficient allowances built in to address biochemical changes associated with disease, inflammation, trauma, malabsorption syndrome, interactions, variations in bioavailability, and stressors.

Feed, Nutrition, Animals

D88 A Comparative Study of the Effect of Four Loko™ Alcoholic Beverages and Other Alcoholic Beverages on Inexperienced Drinkers

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After attending this presentation, attendees will be familiar with the effects of Four Loko™ alcoholic beverage compared to another alcoholic beverage and gain a better understanding of alcohol amounts necessary to become substantially incapacitated.

This presentation will impact the forensic science community by studying the abililty of law enforcement and prosecutorial personnel to successfully investigate and prosecute offenses in which incapacitation by alcohol is a factor.

A local law enforcement agency in rural Missouri initiated an investigation in which a 14-year-old minor female was given Four Loko™ alcoholic beverages, restrained, and orally sodomized. Law enforcement raised the question "Does the Four Loko™ alcoholic beverage affect an inexperienced drinker more than other non-carbonated alcoholic beverages?" Published literature failed to give any definitive insight.

Four Loko™ alcoholic beverages were originally marketed as caffeinated alcoholic malt beverages. Published reports indicate that Four Loko™ no longer contains caffeine, taurine, or guarana. Despite this published literature, Four Loko™ continues to maintain its status among youth as an "energy alcohol." Published research indicates that the effect of Four Loko™ may be due to the delivery system (fruit-flavored carbonation) combined with the alcohol content (12% Alcohol By Volume (ABV)). No published research comparing delivery systems could be located.

This study analyzed both the chemical and behavioral effects on six volunteer test subjects. The volunteers participated in two controlled "wet labs." The labs compared Four Loko™ to vodka and orange juice (commonly referred to as a "screwdriver"), in equal ABV volumes, under similar time constraints. Phase one of the study involved Four Loko™ ingestion while the second involved ingestion of "screwdrivers."

During phase one, subjects were asked to consume one can of Four Loko™ within a 45-minute period. The can was administered in 8-ounce glasses, 15 minutes apart. Intoxication levels were recorded via industry accepted standardized field sobriety tests, breathalyzer tests, vital sign collection, and chemical analysis of blood drawn from the subjects. The test was repeated for a second can. No subject consumed more than two cans. Test subjects were monitored and tested for approximately six hours.

The second, or screwdriver ingestion phase, was conducted one month later and was administered in the same manner as the first phase.

The results of the experiment revealed that intoxication levels were, not surprisingly, similar in equal ABV amounts. The intoxication levels of the test subjects ranged (at peak levels) from .14 to .177. Variation was directly attributable to body habitus. The more telling results were discovered in the social results.

During the Four Loko™ phase, all six test subjects were giggly, happy, and sexually flirtatious. During the first phase, only one of the six test subjects was unable to complete the full battery of testing. This test subject was the most inexperienced drinker of the six test subjects. All six subjects were legally intoxicated after consuming one can of Four Loko™. After ingestion of 1.5 cans (approximately 34-ounces) all six were considered substantially incapacitated.

The social results of second phase were polar opposite of the Four Loko™ phase. Three of six subjects were rendered nearly unconscious after ingestion of approximately 35-ounces of vodka and orange juice (the same volume of liquid as the first phase). Within two hours of ingesting the "screwdrivers" no test subjects were giggly, happy, or flirtatious. The test subject's reactions from screwdrivers were that of a strong desire to lay down and go to sleep, only waking to be administered field sobriety or chemical testing.

The conclusions from this very limited study indicate that while chemically Four Loko™ provides a similar blood or breath alcohol level as other intoxicants in similar ABV concentrations, the social aspects of Four Loko™ make this a product worthy of additional controlled study.

Substantial Incapacity, Four Loko™, Blood Alcohol Concentration

D89 Blast of a High-Pressure Water Pipeline: A Fatal Case With Sternal and Heart Rupture

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After attending this presentation, attendees will understand effects of injuries resulting from a rare work accident of a welder at the local water main management company.

This presentation will impact the forensic science community by showing the causal chain between a high-pressure water jet and thoracic trauma with sternal fracture and heart rupture.

It's the case of a 47-year-old man, who was performing a preliminary inspection to repair a micro-lesion, a few centimeters in diameter, of a high-pressure water pipeline. While the worker was going close to the conduit, a high-pressure water jet widened the gap of the pipeline wall, and squirted through the breach, hitting him in the sternal region, lifting him into the air, and throwing him about 25 meters away, to a lower level with a drop of about five meters. From the testimonies of colleagues, evidence suggests that the subject performed a parabolic flight, swirling with limbs abducted, then landed on his feet, leaving two deep footprints on the ground which were found near the corpse, rebounded, and was found in a supine position.

The external examination didn't show significant injuries except two linear abrasions in the breast region and a compound fracture of the right leg. A full-thickness fracture of the sternum and a fracture of the anterior wall of the left ventricle were discovered during the autopsy. There were also multiple rib fractures on the right rear arch.

Closed thoracic trauma with sternal fractures and visceral injuries may result through three different mechanisms: direct impact injuries, by deceleration, and by compression. Most trauma affects the thoracic wall in a more or less severe way, followed by pleuro-pulmonary injuries; less frequent are heart, diaphragmatic, and esophageal lesions; the rarest are the aorta and great vessels diseases. Sternal fractures are rare. The most common causes are represented by road trauma, involving deceleration injuries, and direct trauma to the anterior chest wall. Indirect mechanisms are the rarest, represented by hyperextension of the trunk or by violent contractions of the muscles of the neck (sternocleidomastoid) and of the trunk, which can occur in several conditions (physical exercises, coughing, vomiting, etc.).

In this case, the damaging mechanism is atypical and seems to be unique in literature: the impact of a high-pressure water jet behaves like a breast injury similar to a direct trauma with a strong damaging power. The found lesions, above all the sternum and the heart, are quite similar and comparable to injuries due to direct trauma caused by a traffic accident or by a direct impact of the chest against a large area.

Bone and visceral lesions are due to the detrimental action of the high-pressure water jet that struck the subject on the anterior chest region. This study presents this case, comparing it with the literature, which was very poor regarding this theme, focusing particular attention on the damaging mechanism that acted on the bony and visceral structures, especially demonstrating the correct causal chain through the ergonomic engineering expert report.

Thoracic Trauma, Heart Rupture, Water Jet

E1 Law and Ethics in End-of-Life Decisions: Where Italy is Going in Comparison With Other EU Countries

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After attending this presentation, attendees will gain insight into the current political and legal dispute, regarding the medical role in end-of-life decisions, giving a schematical account of some cases of deaths that highly impacted on Italian public opinion during the last five years (i.e., Englaro, Welby, Monicelli, Magri) in addition to comparing the trends of others EU countries. In particular, after attending this presentation, attendees will understand: (1) the main juridical and ethical aspects of Italian Law in ELD (End of Life Decisions); (2) the conceptual distinction between direct active and indirect active euthanasia, withdrawal of life-sustaining measures (passive euthanasia), assisted suicide and suicide; and, (3) the statistic data of Italian public opinion regarding euthanasia and the living will, including the acceptance trend in others EU countries.

This presentation will impact the forensic community by highlighting legal similarities and differences among EU countries in the provisions of ELD, stressing the complexity of the relationship between patient (both terminally ill and mentally ill) and physician and trying to enhance the international debate with recent Italian experiences. Exploring international legislation, there are no universal policies regarding ELD nor homogeneous guidelines for physicians faced with ELD issues.

The reconstruction of the examples mentioned above will be presented, taking into account Italian law. Direct active euthanasia, performed by the administration of toxic drugs to a patient with the purpose of inducing his/her death, is explicitly illegal in Italy, and thus punishable either as a homicide (Penal Code article 579) or as voluntary manslaughter (Penal Code article 575) with extenuating circumstances of mercy, even though these extenuating circumstances are not specifically provided for ELD issues. In contrast, indirect active euthanasia, performed by administration of drugs to relieve pain and which may lead to the death of the patient only as a side effect, is not clearly prohibited by the law, rather it may be included among the so-called palliative treatments. Assisted suicide is forbidden by Italian law, being considered a crime of instigation to commit suicide (Penal Code article 580). Similarly, passive euthanasia is also prohibited (Penal Code article 42), although it is perceived by public opinion to be less worthy of punishment. The ethical and legal concerns involving the debates upon ELD reflect the different laws in the other EU countries. Euthanasia and assisted suicide are forbidden in most EU countries. The Netherlands, Belgium, and Luxemburg have legalized euthanasia. Switzerland allows assisted suicide, and recently approved guidelines allow doctors to perform a sort of passive euthanasia in Sweden.

Although the deaths of *Englaro*, *Welby*, *Monicelli,* and *Magri* represent different type ELD cases, they increase public interest regarding ELD issues, in particular euthanasia and the right to decide in advance to give authorization or non-authorization for life-sustaining artificial treatments ("living will"). Those cases are all characterized by the desire to avoid physical and/or psychic pain and poor quality of life. The 2011 Eurispes (Italian Institute of Political Economical and Social Studies) report data will be show that 66.2% of Italians are in favor of euthanasia: 18 to 24-year-olds: 75.3%; 25 to 34-year-olds: 70.9%; 35 to 44-year-olds: 67.7%; 45 to 65-year-olds: 65%; and over 65 years: 53.7%). Additionally, 77.2% of Italians would like a bill that puts "living wills" in writing.

The primary and fundamental issue of ELD is the need of a unifying model that would identify the unique and critical relationship between patient and doctor, whose primary mission should be not only "to cure" but also "to care." It is recommended to focus the attention of forensic community on the relevance that the concept of "quality of life" may have in ELD issues.

End-of-Life Decision, Quality of Life, Patient-Physician Relationship

E2 The Spider's Web Embedded in Competency to Stand Trial: Forced Psychotropic Medication and a Defendant's Right to Due Process to Refuse

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After attending this presentation, attendees will better comprehend the issues surrounding this controversial concept regarding mandated or forced medication and how lawyers, judges, and experts affect a defendant's rights involving due process and loss of liberty concerns.

This presentation will impact the forensic science community by conveying knowledge, competence, and performance about diverse perspectives on these issues that will enable legal practitioners, whether lawyers or judges, to be more competent, aware and knowledgeable in the complexities of this concept for the performance of their duties in and out of the courtroom.

Fascinating legal and medical issues involving a defendant's due process under the Fourteenth Amendment to each person's liberty interest in refusing medication are raised in both criminal and civil contexts. These situations may arise where a court must decide whether a defendant is competent to stand trial and that defendant's refusal of medication asserting loss of liberty. Courts are faced with the delicate balancing which occurs where a defendant's right as a citizen is considered in light of protecting that individual and the community's safety interests. The courts then weigh due process issues while balancing a defendant's right to trial and considering the prosecutor's interests on behalf of the victim in bringing a defendant to trial. After hearing testimony from experts presented by the lawyers or from court-appointed experts, the courts then carry the responsibility to decide whether defendant possesses the capacity to make an intelligent, informed decision about the medication.

Often such competency issues involve having courts decide whether mandated psychotropic medication is necessary. Judges, lawyers, and medical experts struggle with reaching the right or appropriate decision tailored to each defendant's circumstances. Due to the importance of understanding the surrounding medical and scientific concerns, issues involved in the propriety of forced medication can be a nightmare for the judges and lawyers involved in these cases.

Judges and lawyers alike are responsible under the law for knowing what types of resource books to rely upon, selecting an appropriate expert to assist in understanding these technical pharmaceutical and medical issues, and having defendants appropriately evaluated by experts to address the requisite propriety of forced medication. Judges and lawyers in state courts have dealt with these issues in a variety of ways in civil and criminal cases. Judges consider and weigh criteria evaluating risks to determine whether defendant should be committed

due to: (1) substantial risk of physical harm to self or others; or, (2) substantial and immediate risk of serious physical impairment or injury to self; or would benefit from treatment in a hospital for mental illnesses manifested by evidence of behavior creating grave and imminent risks to substantial rights of others or self.

Before permitting courts to order forced medication, legislatures in various state and federal jurisdictions have mandated courts to consider weighing a defendant's vital interests under a variety of analyses such as defendant's (or patient's) best interests and whether a less intrusive treatment exists.

With the complexities of treatment for mental illnesses advancing significantly through the past half-century, professionals are confronted with the dilemma of overestimating the effectiveness of various medications while minimizing the extent of side-effects involved in the medications and possible alternative medications being considered. The presentation will analyze these issues as well as addressing special challenges presented to professionals in the court process where children and parental control issues are involved as well as in the circumstances of sex offenders in involuntary commitment situations.

Forced Medication, Due Process, Competency

E3 Judicial Bias and Scientific Evidence in Criminal Cases

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After attending this presentation, attendees will have a better understanding of the possible predisposition of trial and appellate judges to routinely admit scientific evidence offered by the prosecution in criminal cases.

This presentation will impact the forensic science community by assisting attorneys and forensic scientists in understanding the basic psychological principles that affect judicial admissibility decisions.

What accounts for the current overwhelming court rejection of *Daubert* challenges? Judicial decisions often do not indicate that the judges weighed the scientific validity of proffered evidence meaningfully. Most of the decisions rationalize admissibility based on prior admissions of such evidence. Judges use what has been characterized as ''judicial gymnastics,'' including refusing to hold a hearing, taking "judicial notice" of admissibility, ignoring the *Kumho* case, reversing the burden of proof, or abdicating the "gatekeeper" duty by deferring to jurors to "weigh" scientific issues.

Why? One significant factor may be a systemic pro-prosecution bias on the part of judges that is reflected in admissibility decisions, regardless of the standards of *Frye* or *Daubert*. As Groscup, found, "as a general proposition, judges disfavor civil plaintiffs and criminal defendants and are more likely to rule against them than against their opposites even when presenting equivalent evidence or arguments."[1]

Systemic pro-prosecution bias by judges is a function of elementary psychological concepts. Guthrie described it as a reflection of an "attitudinal blinder," relying on significant empirical studies of judicial attitudes and actions: Whether elected or appointed, judges come to the bench with political views. This is not to say that they have pre-committed to positions in particular cases, but—judges do have opinions, and these opinions or attitudes can predispose them to rule in ways that are consistent with those opinions or attitudes.

To establish the presence of attitudinal blinders among judges, political scientists have developed, and provided empirical evidence to support, the so-called attitudinal theory or model—the evidence suggests that attitudinal blinders are an issue not only at the highest court in the land but also in these lower courts.[2]

These "attitudinal blinders" are especially prevalent in criminal cases and especially in the state courts where most criminal cases are tried. As Professor Rodney Uphoff put it, "In the end, state court judges are, for the most part, rational actors whose attitudinal biases reflect their self-interest and their backgrounds. Most are answerable to a tough-on-crime electorate and are often reluctant, therefore, to make risky political decisions upholding the constitutional rights of criminal defendants."[3] Specifically, Uphoff comments on how this attitudinal bias manifests itself in criminal cases:

Most judges, especially those with prosecutorial experience, presume that most defendants are, in fact, guilty, even though some are, in fact, innocent. This presumption of guilt, pro-prosecution perspective not only affects the manner in which many judges rule on motions, evaluate witnesses, and exercise their discretion, but it also adversely affects the willingness of many judges to police law enforcement agents and prosecutors.[4]

The current legal state of forensic science evidence in criminal cases is somewhat schizophrenic. While many scientists and scholars, and even a congressionally mandated national study, seriously question whether there is validity to non-DNA forensic evidence, trial judges simply continue to admit such evidence and appellate judges continue to affirm them.

References:

1. *Modern Scientific Evidence: The Law and Science of Expert Testimony*, (eds. David L. Faigman, Michael J. Saks, Joseph Sanders, and Edward K. Cheng), 2009-2010 edition, at §1:35, p. 112.
2. Guthrie, Chris, *Misjudging*, 7 Nev. L. J. 420 (2007) at 438-440, citing *The Hearing of Samuel A. Alito, Jr.'s Nomination to the Supreme Court*, Hearing Before the S. Judiciary Comm., 109th Cong. 56 (2006).
3. Uphoff, Rodney J., *On Misjudging and its Implications for Criminal Defendants, Their Lawyers and the Criminal Justice System*, 7 Nev. L. J. 521 (2007), at 532; and see Fredric N. Tulsky, *How Judges Favor the Prosecution*, Mercury News.com (February 12, 2007), available online at http://www.mercurynews.com/search/ci_5128172?IADID=Search-www.mercurynews.com-www.mercurynews.com (last visited December 14, 2011) (claiming that "in a fourth of all jury cases, a review finds, members of the bench apply their tremendous powers in ways that hurt defendants").
4. *Id*.

Judicial Bias, Judges, Admissibility

E4 It Don't Rain in Indianapolis in the Summertime; the Defense Expert is Never Interested in Reliable Scientific Methods; the Government's Expert Will Always Use the Best Methods and Testify From an Unbiased Perspective; and, It Don't Snow in Minneapolis When the Winter Comes

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After attending this presentation, attendees will become aware of the importance of an expert witness to maintain impartiality in testifying.

This presentation will impact the forensic science community by reinforcing the belief that the expert witness must avoid the "desire to win" mentality, which is a product of the adversarial process.

This presentation will examine the experiences of an "expert witness" whose perception of reality is based on decades testifying for the government and whose recognition of reality in the courtroom has faced many "what is happening here" moments.

For someone whose career spanned more than 32 years in forensic science laboratories affiliated with law enforcement agencies, there is an inherent desire to believe in truth, justice, and the American way when entering the courtroom. In the greatest majority of cases, this has and will usually be true. Most forensic science laboratories which are a part of a government organization and which are accredited conform to the requirements for reliability and relevance in following analytical protocols. Many who have spent the entirety of their careers in similar professional settings have come to believe that only the defense will "go after the expert" in the adversarial arena of the courtroom, and then only to keep the client (translation, "bad guy") out of

jail. Now, think *Porgy and Bess* – George Gershwin had it right when he wrote "*Ain't Necessarily So*." The defense does have the legal obligation to cross-examine a prosecution witness in any way deemed appropriate; and the prosecution does have a legal obligation to cross examine a defense expert to demonstrate the invalidity of contrary opinions. Winning at any cost is not supposed to be a part of the equation; however, sometimes this appears to be exactly what is happening.

The reality is that on both sides, there are always elements in any expert witness testimony which can and should be determining factors in distinguishing between what "one claims to see" (sometimes with one eye shut) from "what is." The courtroom is not always a search for the truth; and the adversarial system does influence priorities in ensuring that the process gets it right. Both sides at times start with a conclusion and then work backwards in considering only those data interpretations that supports these pre-determined conclusions. This is the scientific method in reverse. Testing the hypothesis is supposed to precede the formulation of a conclusion. There are still expert witnesses who refuse to acknowledge the fact that standardized methodology and requirements for documentation do exist, and everyone who testifies as an expert witness, especially witnesses called by the government, must conform to these standards. There are still too many government witnesses who use a cafeteria style approach to testifying as an expert. All of this and more happens on both sides in the courtroom, both pre-trial and then again during the trial. There are factors both the defense and especially the prosecution can and should consider in determining the reliability, relevance, validity and veracity of expert witness testimony:

1. The government seeks expert witness testimony outside of a government entity when the expertise exists within that entity.
2. The expert witness admits that the government retained their services to state a specific opinion.
3. The analytical approach to evidence analysis is "holistic" rather than method specific.
4. There are no documented protocols or evidence of conformance to standards.
5. The expert witness bases conclusions on "training and experience" rather than on empirical data or demonstrative evidence.
6. A laboratory which loses accreditation or which does not meet the requirements for extending accreditation has serious problems.
7. Referencing the internet as the only authoritative source is questionable at best.

These situations not only "can happen," they have all been experienced in a very narrow timeframe of two years. In the courtroom, the judge, as "gatekeeper," makes every attempt to ensure that expert witness testimony meets the standards of the jurisdictional rules of admissibility. However, that gatekeeper's responsibility and the responsibilities of all participants in the process require more than a belief that the "good guy always wears a white hat." Everyone who testifies in a courtroom has a responsibility to play by the rules, irrespective of where the subpoena to appear originates, especially when the expert is testifying "for the prosecution" in a criminal trial. This does not always happen.

The desire to win is a product of the adversary system, and this adversarial process can usually be very effective at arriving at a just verdict. However, arguments based on challenges to methodology, documentation, data, and the validity of conclusions can be more persuasive than the occasional personal attacks from both sides during the "adversarial processes" in the courtroom.

Expert Witness, Standards, Adversarial Process

E5 The Effect of the NAS Report Upon Courts in Admitting Forensic Expert Testimony

Joseph J. Maltese, JD, New York Supreme Court, 130 Stuyvesant Pl, 3rd Fl, Staten Island, NY 10301*

After attending this presentation, attendees will gain an understanding of the extent of the impact the NAS Report recommendations have had on judges deciding whether to preclude or limit expert witness testimony.

This presentation will impact the forensic community by providing the results of federal and state court decisions that have referenced the NAS Report, and to what extent certain types of forensic specialists have been precluded or limited in testifying in court.

In February of 2009, the National Research Council of the National Academy of Sciences (NAS) published their long-awaited Report entitled, "*Strenghthening Forensic Science in the United States: A Path Forward*," which is commonly refered to as NAS Report.[1] The NAS Report reviewed: the fundamentals of the scientific method as applied to forensic practice, such as hypothesis generation, testing, falsifiability, replication and peer review of scientific publications. The Report also assessed the methods and technologies of forensic science such as: the collection and analysis of forensic data; the accuracy and error rates of forensic analysis; the sources of potential bias and human error in the interpretation by forensic experts; and the proficiency testing of forensic experts.

The NAS Report, made 13 recommendations. The first and primary recommendation was to "promote the development of forensic science into a mature field of multidisciplinary research and practice," founded on the systematic collection and analysis of relevant data. In order to effectuate that over-riding recommendation, the report recommended that Congress should create a National Institute of Forensic Science (NIFS) to focus on establishing "best practices" for forensic science professionals and forensic laboratories. Once established and funded, the NIFS should establish standards for mandatory accreditation of forensic science laboratories, scientists, and medical examiners. The report recommended that NIFS should promote scholarly, competitive peer-reviewed research, and technical development in the forensic sciences. It should also develop strategies to improve forensic science research and educational programs and new technologies. Lastly, it should develop programs to improve understanding of forensic science disciplines and their limitations within the legal system.

While the Congress has not appropriated monies to fund the National Institue of Forensic Science, the NAS Report with its critique of certain forensic scpecialties has been presented to judges by attorneys in court proceedings as part of their applications to preclude certain expert witnesses, or at least to limit the extent of their testimony and opinions before the judge or jury. This study will review the 25 federal and 26 state court published decisions that have cited the NAS Report from Febrauary 2009, when the NAS Report was first published until August 1, 2012, to ascertain what impact, if any, the report has had on the court decisions to preclude or limit expert testimony.[2] The study will evaluate whether courts are merely citing to the NAS Report to acknowledge its existence, or is the NAS Report cited as a rationale for a court's decision to preclude or limit expert witness testimony.

In conclusion, this study will provide some insight into the impact the NAS Report has had on some court decisions on the admisssibility of some forensic specialties and how the forensic community can address those issues the courts found significant in the future.

References:

1. National Research Council of the National Academies, *Streghthening Forensic Science in the United States: A Path Forward*. Washington, DC: National Academies Press, 2009.
2. Westlaw query: "Streghthening Forensic Sciences in the United States," All case Data Base (accessed August 1, 2012).

NAS Report, Experts, Admissibility

E6 Federal Criminal Discovery

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The goal of this presentation is to explore how the attorneys in the process can facilitate the proper transfer of discovery material.

The FBI Laboratory at Quantico processes thousands of cases every year. Many of these requests come from state agencies. Ultimately, laboratory examiners and FBI case agents may testify in state criminal trials. The interplay of federal criminal discovery in state trials varies as a result of multi-jurisdictional local rules. However, all discovery must satisfy the inherent rules of *Brady* and *Giglio*. The role of the attorneys in requesting proper discovery material and delivering or achieving the receipt of this information is very important. How such information is handled and provided to parties is inherently critical to the judicial process. This presentation will impact the forensic science community by examining the varying roles and duties of the attorney from the generic framework down to the specific concluded cases including Casey Anthony, Santae Tribble, and Kirk Odem.

Scientific evidence is a critical and crucial element in seeking the just resolution in criminal case proceedings. Federal laboratories, for example, the FBI Laboratory at Quantico, Virginia, the ATF&E Laboratory in MD, process thousands of cases every year involving evidence associated with federal and state criminal investigations. As a result, federal laboratory examiners may be called upon to produce discovery documents and testify in both federal and state criminal proceedings. The interplay of federal criminal discovery responsibilities in producing discovery material to state attorneys is further complicated as a result of multi-jurisdictional local rules which direct or provide guidance to state prosecutors regarding what must be provided to opposing counsel. However, all discovery must satisfy the inherent rules of *Brady v. Maryland, 373 U.S. 83 (1963*) and *Giglio v. United States*, 405 U.S. 150 (1972) which require prosecutors disclose exculpatory evidence or information that impeaches the credibility of government witnesses against a defendant. The role of the attorneys, as the proponent in offering the scientific evidence and associated testimony, in requesting proper and comprehensive discovery material, the laboratory's role in providing all relevant documents; and the delivery these documents by the government to the defense is inherently critical to facilitate a fair and accurate process that convicts the guilty and sets the innocent free.

More than three decades after a rape victim identified Kirk L. Odom based on eyewitness testimony, new DNA testing of evidence, a hair fragment, stains on a pillowcase, and robe confirmed Odom's innocence. The U.S. Attorney in the case in a court filing stated that "More than 30 years after Mr. Odom's conviction, DNA testing reveals that he suffered a terrible injustice. The United States expresses its profound regret for the harm suffered by Mr. Odom, and requests that this court immediately vacate Mr. Odom's convictions and dismiss the indictments against him with prejudice." In 1978, Santae Tribble was accused of murder, in part, based on a single hair that supposedly matched Tribble's "in all microscopic characteristics" from a stocking worn by the killer. In 2012, federal prosecutors moved to have Tribble's 1980 conviction vacated based, in part, on this testimony and subsequent scientific testing of the case evidence. What discovery obligations attach to any scientific testing conducted by either the government or the defense on the evidence?

From the time a defense attorney seeks discovery, for example, in a case such as *Florida v. Casey Anthony*, or begins a new challenge based on scientific evidence, what is the interplay between the forensic laboratory, the prosecutor (federal or state), and the defense? If a laboratory determines that prior scientific testing of case evidence was incorrect, how does that information get transferred to the relevant parties: the defense attorney, defendant, and the courts? How do judges view the exchange of discovery? What are the legal and ethical requirements for all involved?

This panel presentation will explore these cases and other contemporary issues involving: (1) new scientific testing of old cases and how the attorneys in the process can facilitate the proper transfer of discovery material; (2) how judges are the gatekeepers of the search for the truth; and, (3) the varying roles and duties of the attorney from the generic framework down to the specific concluded cases including cases the panelists have been involved in including those of Casey Anthony, Santae Tribble, and Kirk Odom.

Casey Anthony, FBI Lab/Quantico, Federal Criminal Discovery

E7 The Right of Being a Donor Until the Real Death and Beyond

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After attending this presentation, attendees will understand the differences of opinion and legislation among Italian and European Union (EU) and other EU countries in the field of organ transplants as well as the definition of death.

This presentation will impact the forensic community by analyzing social, ethical, and legal aspects among different nations in the provision of organ transplants, taking into account rare aspects concerning the definition and determination of death, procedures of organ donation, and consent/right of being a donor.

This report reviews the ongoing debate concerning organ transplantation in Italy, comparing the Italian point of view with other countries'. Currently, the challenge in the EU regarding organ transplantation is to increase the supply of donors and ensure the quality and safety of the process from donation to transplantation. Therefore, in December 2008, the European Commission formulated several priorities to be enacted in order to strengthen the cooperation among Member States. These priorities were included in the "Action plan on Organ Donation and Transplantation (2009-2015): Strengthened Cooperation between Member States." The current legislation in Italy (Law 578/1993 - DM 2008) accepts two different assessments of death: an observation period of six hours with two checks, using EEG at the beginning and at the end of the period or, alternatively, continuous ECG for at least 20 minutes. Despite the obvious limitations imposed by the criteria of determination of death, the quality of the performed transplants has improved significantly with transplant outcomes comparable or superior to the main European countries (as evidenced by the main international registries). This achievement has been confirmed by a detailed study performed by the Italian Superior Health Institute (ISS - the leading technical and scientific public body of the Italian National Health Service) that, in line with European goals, has initiated a project for assessing the quality of health care with the aim of improving health conditions, raise the level of satisfaction of citizens and improve transparency.

Since 2008, due to the application of experimental protocols adopted by some leading Italian hospitals, the damages caused by prolonged cardiac observation period (20 minutes) have been minimized. These protocols are conceived to optimize logistics planning for the early identification of potential Non-Heart-Beating Donors (NHBDp). The NHBDp is a subject which doctors should consider the possibility to become a NHBD, before the assessment of clinical and legal death. The NHBDp category is essential in the organization of health care, and is compliant with legal and ethical principles. The organs preservation should be started very early (from the moment there is a possibility of evolution from a NHBDp into NHBD), applying all

actions and techniques suitable for this purpose. However, always respecting the consent and (if applicable) the status of a living person, and the dignity of the corpse. Without taking these preventive actions, that are put in place while waiting the assessment of the individual's will or, alternatively, the non-opposition of relatives or legal representative (pursuant to Law 91/1999), the medical staff may infringe the right to donate organs (or the principle of non-opposition). Therefore, it would be contrary to the purposes of the law and to the respect due to the will of the donor, not to guarantee his right of being a donor on technical grounds.

In conclusion, although Italy does not implement the provisions of the Protocol of Pittsburgh, adopted by several countries (such as US, ES, NL), we are able to obtain very encouraging results in terms of transplants thanks to maximum optimization of all the technical, organizational and logistical aspects of the organ donation process.

Organ Transplantation, Informed Consent, Death

E8 NSTC Subcommittee on Forensic Science Process and Path Forward for U.S. Crime Laboratories: A Voluntary, Consensus, Based Approach

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After attending this presentation, attendees will learn of the final recommendations of the Subcommittee on Forensic Science for ongoing improvements of forensic science, learned about a new federal Executive Branch initiative and discussed potential strategies for uniform implementation of the recommendations.

This presentation will impact the forensic science community by stressing how implementation strategies for the recommendations and the new federal initiative may dramatically affect forensic science laboratories, experts, accreditation and certification bodies, and standards development. The implementation strategies for the Subcommittee's recommendations and the new federal initiative may have a dramatic effect on forensic science service providers, experts, accreditation and certification bodies, and standards development methodologies. The National Academy of Sciences (NAS) Report, "*Strengthening Forensic Science in the United States: A Path Forward,"* called for change and significant improvement in the forensic sciences. The Federal government, the courts, law enforcement, and the forensic science enterprise as a whole have worked diligently to develop a meaningful response to the NAS report. The culture of enhanced scientific, quality, and integrity continues to evolve and improve. The question is how will forensic scientists embrace it?

In 2009, the Office of Science and Technology Policy, under the auspices of the National Science and Technology Council, established the Subcommittee on Forensic Science (SoFS). The SoFS's primary mission was to assess practical challenges and make recommendations associated with the findings of the NAS Report. Over the last three years, SoFS actively pursued the investigation and analysis of critical issues which can inform a coordinated and meaningful advancement of the concepts enunciated in the NAS Report through the SoFS five interagency working groups composed of Federal, state, and local laboratory representatives, academics, lawyers, judges, researchers, and law enforcement officials. The SoFS's detailed and comprehensive exploration has broadened the breadth of foundational knowledge and situational awareness, thereby informing a meaningful framework for moving forward. Now that the SoFS has published its recommendations, its successor entity is ready to provide sustainable leadership and direction to coordinate uniform implementation within the United States.

This presentation will discuss implementation strategies for the specific recommendations issued by the SoFS that may affect many forensic science service providers in the U.S. Foremost is a voluntary, consensus-based approach through a federal coordination body. Other strategies also exist; however, such as federal legislation, state legislation, state forensic science commissions that range in authority from advisory to regulatory, and uniform state laws created through a consortium of state governments. The vehicles for and challenges and alternatives to wide-spread, uniform implementation will be explored by the panel. Strategies for implementation and enforcement must be considered to promote quality and ensure the scientific integrity of forensic science. This panel will discuss the opportunities and challenges associated with implementing SoFS recommendations. Issues such as accreditation, certification, education, proficiency testing (and proficiency test providers), scientific working groups, vocabulary, report writing, uniform code of ethics, medicolegal death investigators and other matters will be discussed. Consideration will also be given to the role of the federal and state governments in supporting implementation of the recommendations, the role of discipline-specific working groups in forensic science standards development, the role of accreditation and certification bodies in enforcing the recommendations, and the role of each forensic science service provider in ensuring the continual improvement of forensic science in the United States.

Recommendations, Subcommittee, Federal Initiative

E9 ABA's Proposed Changes to Legal Practices That Impact Expert Testimony

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After attending this presentation, attendees will learn about the American Bar Association's recommendations on how to change legal practices to shape the interaction of law and forensic science regarding admissibility of evidence, questioning of and instructions to jurors regarding scientific evidence, contents of laboratory reports, uniform vocabulary, as well as what ethics lawyers should expect from forensic scientists they hire.

Forensic science is the application of science to the law, and is therefore dependent upon the legal system's procedures for admissibility of evidence, discovery requirements, juror education, and establishing ethics for expert witnesses. The American Bar Association is the most influential organization representing lawyers and judges in the United States. Its views and recommendations must be taken seriously by the forensic science community. Every practicing scientist will want to attend this presentation to learn what changes they may expect next time they appear in court. This presentation will impact the forensic science community by pointing out changes on the horizon, as the American Bar Association's views and recommendations must be taken seriously by the forensic science community.

The American Bar Association, America's largest organization representing attorneys, has passed several resolutions calling on lawyers and judges to change their practice and procedure in the courtroom. These resolutions will impact laboratories and the testimony of forensic scientists. Included in these resolutions are:

RESOLUTION #101, urging federal, state, territorial, and local governments to adopt pretrial discovery procedures requiring laboratories to produce comprehensive and comprehensible laboratory and forensic science reports for use in criminal trials to include identification of:

1. The procedures used in the analysis.
2. The results of the analysis.
3. The identity, qualifications, and opinion of the analyst.

4. The identity and qualifications of those who participated in the testing including peer review or other confirmatory tests; and,
5. Any additional information that could bear on the validity of the test results, interpretation or opinion.

RESOLUTION #101C, urging judges and lawyers to consider the following factors in determining the manner in which expert testimony should be presented to a jury and in instructing the jury in its evaluation of expert scientific testimony in criminal and delinquency proceedings:

1. Whether experts can identify and explain the theoretical and factual basis for any opinion given in their testimony and the reasoning upon which the opinion is based.
2. Whether experts use clear and consistent terminology in presenting their opinions.
3. Whether experts present their testimony in a manner that accurately and fairly conveys the significance of their conclusions, including any relevant limitations of the methodology used.
4. Whether experts explain the reliability of evidence and fairly address problems with evidence including relevant evidence of laboratory error, contamination, or sample mishandling.
5. Whether expert testimony of individuality or uniqueness is based on valid scientific research.
6. Whether the court should prohibit the parties from tendering witnesses as experts and should refrain from declaring witnesses to be experts in the presence of the jury.
7. Whether to include in jury instructions additional specific factors that might be especially important to a jury's ability to fairly assess the reliability of and weight to be given expert testimony on particular issues in the case.

RESOLUTION #101D, urging urges judges and lawyers to consider the following factors in formulating jury *voir dire* in criminal cases where forensic science evidence is contested:

1. Jurors' understanding of general scientific principles, including specialized training in science, knowledge or education in science and experience with laboratory practices.
2. Jurors' understanding of specific scientific principles relevant to the forensic science evidence that may be presented at trial, including specialized training, knowledge or education in the specific scientific discipline utilized in the case [chemistry, toxicology, engineering, etc.].
3. Any preconceptions that jurors may have about the forensic science evidence; and,
4. Jurors' bias for or against scientific evidence, including whether scientific results will be accepted or rejected without consideration.

PROPOSED RESOLUTION #300, urging counsel to utilize proposed Guidelines for Conduct of Experts Retained by Lawyers. These Guidelines pertain to Integrity/Professionalism; Competence; Confidentiality; Conflicts of Interest and Disclosure; and Contingent Compensation.

To assess the viability and impact of these resolutions, an expert panel of lawyers and both public and private forensic scientists will discuss the recommendations.

ABA, Admissibility, Legal Practices

E10 The Supreme Court's Decision on *Daubert* After 20 Years

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After attending this presentation, attendees will appreciate how the U.S. Supreme Court's landmark decision in *Daubert v. Merrell Dow Pharmaceuticals* (1993) has developed over the last 20 years.

This presentation will impact the forensic science community by explaining the transformation of the *Daubert* decision from one that many believed lowered the barriers to the admissibility of expert testimony to one later described by the Supreme Court as imposing an "exacting standard."[1]

Daubert and its progeny revolutionized the way courts decide the admissibility of expert testimony. Prior to *Daubert*, the majority of courts in this country, both state and federal, followed the "general acceptance" test for determining the admissibility of scientific evidence. This test was derived from *Frye v. United States*, a 1923 decision of the D.C. Circuit. *Daubert* rejected the *Frye* test as a matter of statutory interpretation; however, from its beginning, lower courts struggled to interpret the *Daubert* opinion. Numerous passages in the opinion suggested that more evidence should be admissible under *Daubert's* new reliability test than under the *Frye* general acceptance test, which the Court rejected. Yet, over time, the judiciary's "gate keeping" role under *Daubert* developed into a stringent test—at least in civil cases. "The Federal Judicial Center conducted surveys in 1991 and 1998 asking federal judges and attorneys about expert testimony. In the 1991 survey, seventy-five percent of the judges reported admitting all proffered expert testimony. By 1998, only fifty-nine percent indicated that they admitted all proffered expert testimony without limitation. Furthermore, sixty-five percent of plaintiff and defendant counsel stated that judges are less likely to admit some types of expert testimony since *Daubert*."[2]

In contrast, *Daubert* did not have the same effect in criminal litigation. In 2000, one commentator noted, "the heightened standards of dependability imposed on expertise proffered in civil cases has continued to expand, but expertise proffered by the prosecution in criminal cases has been largely insulated from any change in pre-*Daubert* standards or approach."[3] In addition, an extensive study of reported criminal cases found that "the *Daubert* decision did not impact on the admission rates of expert testimony at either the trial or appellate court levels."[4]

The disparate treatment of federal civil and criminal cases has been criticized, including in the National Academy of Sciences 2009 Report on forensic science. After noting that "trial judges rarely exclude or restrict expert testimony offered by prosecutors," the report commented: "The situation appears to be very different in civil cases. Plaintiffs and defendants, equally, are more likely to have access to expert witnesses in civil cases, while prosecutors usually have an advantage over most defendants in offering expert testimony in criminal cases. Ironically, the appellate courts appear to be more willing to second-guess trial court judgments on the admissibility of purported scientific evidence in civil cases than in criminal cases."[5] The report went on to conclude: "The bottom line is simple: 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*." *Id.* at 53.

The cause of this disparity remains disputed, as does the advantages of *Daubert* when compared to *Frye*. A dozen or so states have retained the general acceptance test. Because many of these jurisdictions are populous (e.g., California, New York, Florida), *Frye* remains important.

Yet, *Daubert's* impact on forensic science has been substantial. Along with the advent of DNA in the late 1980s, *Daubert's* emphasis on empirical testing resulted in a paradigm shift in the treatment of expert testimony in criminal trials. Within two years of *Daubert*, a courtroom challenge to the admissibility of handwriting evidence had occurred. These were followed by attacks on fingerprint and then firearms identification comparisons. Although these attacks have had limited success, they did expose the lack of an empirical bases for many common techniques. Moreover, these challenges would not have occurred under the *Frye* test. Interestingly, other techniques, such as bite mark and hair comparisons seem immune from attack under *Daubert*.

References:

1. *Weisgram v. Marley Co*., 528 U.S. 440, 455 (2000).
2. Berger, *Upsetting the Balance Between Adverse Interests: The Impact of the Supreme Court's Trilogy on Expert Testimony in Toxic Tort Litigation*, 64 Law & Contemp. Probs. 289, 290 (2001).
3. Risinger, *Navigating Expert Reliability: Are Criminal Standards of Certainty Being Left on the Dock?*, 64 Albany L. Rev. 99, 149 (2000).
4. Groscup et al., *The Effects of Daubert on the Admissibility of Expert Testimony in State and Federal Criminal Cases*, 8 Pyschol. Pub. Pol'y & L. 339, 364 (2002).

5. Nat'l Research Council, Nat'l Acad. of Sci., *Strengthening Forensic Science in the United States: A Path Forward* 11 (2009).

***Daubert* Decision, Admissibility, Expert Testimony**

E11 Anatomy of a Crime Lab: How Even a Lawyer Can Investigate the Reliability of Test Results

Christine Funk, JD, and Lauri Traub, JD*, 919 Vermillion St, Ste 200, Hastings, MN 55033*

After attending this presentation, attendees will understand basic tools with which to arm themselves when investigating the reliability of forensic science intended to be offered in court.

This presentation will impact the forensic science community by demonstrating the importance of investigating the conclusions provided to lawyers by forensic scientists. It will provide prosecutors and defense attorneys with a basic road map to face the challenge of assuring foundational reliability of evidence offered in court.

In the spring of 2012, a lawyer with an undergraduate degree in business administration sought to learn more about the test results offered by the Saint Paul Police Department Crime Lab in a case involving drug chemistry. With the assistance of a lawyer with a Bachelor of Arts in Social Work, a request for the underlying file was made. Reviewing the file, much of it made no sense whatsoever. An appointment was made to meet with the analyst to review the file. At the time the appointment was made with the analyst, the lawyers expected nothing more than to have the science of drug testing and the contents of the testing file explained to them. The thought that something was very wrong in the St. Paul Police Department Crime Lab never entered their minds. The attorneys notified the prosecutor of the meeting as a professional courtesy. The meeting with the lab analyst lasted a little more than an hour and was memorialized in notes taken by the prosecutor.

In very short order, questions about the file were raised – including issues of record keeping, the lack of the use of blanks, deviations from protocols, and the absence of validation studies.

The lawyers left the meeting with more questions than answers. Using SWGDRUG minimum guidelines, the lawyers returned to the lab. In a meeting with the lab director, the lawyers became more alarmed. The lab director had taken the position that SWGDRUG guidelines were just that, guidelines, and therefore they did not have to follow them.

Armed with this information, a meeting was scheduled with a local expert. His response? "This is Houston all over again." He agreed to work on the case and spent countless hours teaching two attorneys the science of drug testing.

This presentation will include an overview of the history of this and other crime lab failures. Some of the fault can be laid at the feet of crime labs operating outside generally accepted practices; however, some of the fault must also be ascribed to the judges tasked as gatekeepers, prosecutors who for years introduced reports without question, and defense attorneys who lacked the time, knowledge or motivation to investigate.

Determining what witnesses should be called, what documents should be introduced, how to work with your experts to learn the science and effectively cross-examine the witnesses and what testimony should be sought in a hearing to challenge the admissibility of evidence will also be explored.

Frye-Mack, Drug Testing, St. Paul Crime Lab

E12 Forensic Chemistry and Toxicology Laboratory Tests: Are Reliable Principles Being Reliably Applied?

Glenn Hardin, MPH, Hamline Univ, Forensic Sciences Program, 1536 Hewitt Ave, MB 239, St. Paul, MN 55104-1284*

After attending this presentation, attendees will understand the principles that make forensic chemistry and toxicology laboratory test results reliable, what laboratory documents are necessary to assess whether those reliable principles have been reliably applied to the case at hand, and how to evaluate the requested documents to determine if the reported results are, in fact, reliable.

This presentation will impact the forensic science community by describing the importance of examining all relevant laboratory documents. Attorneys play a critical role in the criminal justice system since they are the last reviewers before the trier of fact evaluates the physical evidence. To be effective in this role, knowledge of what documents to request and what documents demonstrate reliability, skills in how to evaluate the documents, and understanding to recognize when to consult with an expert.

Why it is that so often attorneys accept crime laboratory reports at face value and fail to request critical documents? Most testing methodologies used in forensic chemistry and toxicology are decades, if not centuries, old. As methodologies, the principles of screening tests (color, microcrystal, and immunoassay tests) are reliable; the principles of confirmation tests (gas chromatography-mass spectrometry (GC/MS), liquid chromatography-mass spectrometry (LC/MS), and infrared (IR)) are reliable; and, the principles of determining sample weights using analytical balances are reliable. However, reliable principles must be reliably applied. Critical laboratory documents hold the key to determining if the crime laboratory's reported results are reliable.

Naming practices for laboratory documents vary from state to state and laboratory to laboratory. Familiarity with a laboratory's nomenclature is crucial. Attorneys sometimes use boilerplate request-for-discovery letters that contain lists of documents that were drafted by an attorney in a different state. Often much of what is requested is not provided by the laboratory, as the laboratory uses different language to describe their documents. If requesting attorneys don't understand what they are requesting and why, they may not realize that they are not getting the documents they need to properly evaluate the physical evidence. If a red document is requested, one that the laboratory calls a fuchsia document, the laboratory probably won't provide the desired document and they won't inform the requestor why the requested documents are not being provided.

The reliability, or lack thereof, of scientific findings affects criminal, civil, and family court matters. Cases will be presented illustrating how reliability can turn on large details and seemingly small ones as well.

Small elements that can result in a laboratory result being excluded include a police officer's missing signature on a breath-alcohol test report, a missing internal (laboratory) sample chain of custody, or a failure to demonstrate analytical sensitivity through proper use of calibrators and controls. Large occurrences that can affect a laboratory result include a lack of written laboratory protocols, training records, bench notes, and method validation. Evaluation of the documents of reliability can lead to a sample result being excluded or in some cases —shutting down an entire laboratory.

Reliability must be demonstrated for every test on every item of physical evidence in every case. Reliability can only be demonstrated through documents.

Reliability, Evidence, Testing

E13 An Independent Forensic Laboratory May Reduce Bias

Everard J. Cowan, PhD, Fairleigh Dickinson Univ, Institute for Forensic Science Administration, 285 Madison Ave, M-MS1-03, Madison, NJ 07940*

After attending this presentation, the attendees will understand and be able to apply the four forensic science laboratory design principles: independence, democratic and local management, scientific inquiry, and scientific truth, also taking into account the tight relationships among the prosecution, police, and crime labs may introduce bias into forensic analysis. Attendees will learn to appreciate the importance of who the boss is. Attendees will understand how the alignment among the three organizations is created by having the same objectives, e.g., maximize number of convictions; by who funds the laboratory, determines and measures the success of the lab directors or supervisors; and by the integrated culture created among the prosecution, police and forensic scientists. Finally, attendees will learn the basic principles concerning how to change this relationship to minimize bias and to apply the design principles to redesign an organization. They will learn that these principles may require changing the principal (the boss) and the incentives leading to the crime laboratory identifying with the prosecution or police. They will learn that laboratories may need to report to a board of directors, rather than the prosecutor and police; may need to develop compensation plans for middle managers to encourage them to build a culture of learning based on error tolerant systems rather than error punishment; and may need to facilitate identity changes by the middle managers to encourage them to engage in the necessary organizational changes.

This presentation will impact the forensic science community by ensuring the lab is independent of the prosecution or defense and by describing monetary and non-monetary incentives that focus on producing scientific truth, leading to support a criminal justice system that will be seen as fair and impartial.

This presentation suggests that creating an independent forensic laboratory may reduce bias caused by the current tight relationship among the prosecution, police, and crime lab. When crime laboratories are funded by and aligned with the objectives of the prosecution or police, there is an increasing probability of conviction. This relationship may create the incentive to convict, with the matter of guilt or innocence as a secondary matter. This incentive to convict may induce unconscious bias in forensic-science analyses and interpretations. Labs that are independent of the prosecution and police (and the defense) will tend to be less subject to unconscious bias and bias-induced errors.

Who the boss is, is important. In any organization, it is the boss who sets the agenda, determines what success is and how that success is measured and rewarded. Organizational economics refers to this as a principal-agent relationship. The principal, in the tight relationship among the prosecution, the police, and the forensic laboratory, is either the prosecution or the police. The agent is the forensic laboratory. The direct agents are the directors and laboratory supervisors, i.e., the middle managers. The agents are motivated by monetary and non-monetary incentives. These incentives encourage the managers and the scientists to identify with the objectives of the principal. In a tight-knit relationship, the objectives of the principal and agent are aligned. In the criminal justice system, an objective is to achieve a high conviction rate. The prosecution, police, and crime lab are aligned in this objective. Incentives are built around this objective. This leads to strategic screening of who to bring to trial, what evidence to process through the crime lab and what evidence to present to decision makers, the judge, or jury. If errors are not made, then this process would be acceptable.

Unfortunately, errors of both commission and omission have occurred and will, in all likelihood, continue to occur. The tight relationships among the prosecution, police and the crime lab ensure that strategic screening will continue to occur; bias will creep into cases; and errors, convicting the wrong person or freeing the actual perpetrator, will continue. This paper proposes to level the playing field between the prosecution and defense and to ensure that the crime laboratory delivers unbiased scientific evidence to both the prosecution and defense witnesses.

Organization, Bias, Independent

E14 How Do Confrontation Clause Requirements Apply to Crime Laboratory Reports? Effective Direct and Cross-Examination of Forensic Scientists Following Williams vs. Illinois, 567 U.S. ___, 132 S.Ct 2221 (2012)

Winona J. Agbabiaka, JD, Brian J. Walsh, JD*, and Anne C. Petty, JD*, Office of the Cook County, Public Defender, 69 W Washington, 17th Fl, Chicago, IL 60602*

After attending this presentation, attendees will understand the current developments regarding presentation of expert witness testimony, where the witness bases his or her opinion on the reports of a non-testifying expert.

This presentation will impact the forensic science community by facilitating improvement of communication of scientific evidence by prosecutors and defense attorneys to the courts in keeping with the requirements of the Constitution. This will help lawyers communicate to judges and jurors both the capabilities and the limitations of forensic science issues to fully present and challenge the methods used to arrive at expert opinions.

Judges, lawyers, and laymen have come a long way in understanding and entering into the world of forensic science. However, there is a need for additional research and education in order to avoid the common misconceptions of forensic science infallibility and absolute certainty and to ensure that the constitutional protections of criminal defendants including the right of confrontation and the prosecution's burden of proof are not diminished or given short shrift. Numerous publications have addressed the misconception that DNA evidence has a special aura of certainty and mystic infallibility.

Despite these misconceptions, prosecutors have a duty and a responsibility to safeguard the constitutional rights of all citizens including the defendant and their preeminent goal is not victory, but justice.[1] Defense attorneys have a duty to engage evidentiary rules to shield their client from a decision based on unreliable evidence and to appreciate and understand the legal principles applicable to the case.[2]

Several recent United States Supreme Court decisions have addressed the application of the constitutional duties imposed on the prosecution and defense in the area of forensic science testimony. The decision in *Williams v. Illinois* is a 5-4 plurality decision in which a majority of the Court agrees with the result, but not the rationale of the opinion. The decision, concurrences and dissent present several different approaches to the issue of what the Constitution requires when expert witness testimony is at issue in a criminal trial.

Which approach should be followed in court proceedings involving the use of expert witness testimony following the Court's decision in *Williams v. Illinois*? The four main perspectives provided by the Court will be discussed. The main approaches proposed by the decision and dissent include: (1) the reports of non-testifying experts relied on by testifying experts are not offered for the truth of matter asserted and are not subject to the confrontation clause; (2) the lab report is not testimonial and is therefore presumptively admissible; however the defense may call the lab witnesses if they are available. Moreover, should the defense show good cause to doubt the competence of the laboratory producing the report or the validity of its accreditation, then the accused would be entitled to confrontation clause protection; (3) the report is offered for the truth of the matter asserted therein, however it is not testimonial and thus does not violate the confrontation clause; and, (4) prior Supreme Court decisions should govern, find that lab reports of non- testifying experts relied on by expert witnesses are indeed testimonial and should not be admitted in evidence without allowing

cross-examination of the persons who performed the testing and prepared the reports.

Although the discussion and litigation of these issues is not settled; judges, prosecutors and defense attorneys must determine how to proceed in keeping with their respective constitutional duties and responsibilities. This presentation will focus on which witnesses should be called to testify regarding a specific DNA report and why.

References:

1. *People v. Weilmuenster*, 283 Ill. App. 3d 613, 626**;** 670 N.E.2d 802, 810 (2nd Dist. 1996).
2. *People v. Watson*, 2012 Ill. App. (2d) 91328 par 22; 965 N.E. 2d 474, 481; 358 Ill. Dec. 403.

Expert Testimony, Confrontation Clause, Forensic Science

E15 Forensic Expert Testimony Meets the Confrontation Clause

James K. Record, LLM, California Univ of PA, Dept of Justice, Law & Society, 250 University Ave, California, PA 15419*

After attending this presentation, attendies will understand how recent U.S. Supreme Court decisions have impacted forensic expert testiomony.

This presentation will impact the forensic science community by explaining how recent U.S. Supreme Court decisions concerning forensic experts and the confontation clause directly affect participation in the criminal justice system.

There is no setting where credible and reliable testimony is more essential than that of the forensic expert in a criminal case. *Williams v. Illinois,* is the latest U.S. Supreme Court decision involving the application of the confrontation clause to forensic evidence.[1] It involved a forensic analyst testifying, in part on a DNA profile performed by someone else, that DNA found inside a rape victim matched DNA taken from the defendant. To understand the issue this fact-pattern presented, it is necessary to give a brief background.

The confrontation clause guarantees the accused the right "to be confronted with the *witnesses* against him." Because "witnesses" are people to give testimony, a broad coalition of Justices held in *Crawford v. Washington,* that the confrontation clause prohibits the prosecution from introducing out-of-court "testimonial" statements without putting the declarants on the stand.[2]

In *Melendez-Diaz v.* Massachusetts, the Court held that forensic reports that certify incriminating test results are testimonial.[3] The case, however, was a closely fought five-to-four decision and last term, in *Bullcoming v. New Mexico*, a five Justice majority reaffirmed *Melendez-Diaz* and made clear that when a prosecution wishes to introduce a certified forensic report, it does not suffice to call a supervisor or other "surrogate" witness to the stand in the place of the actual author of the report.[4]

The *Bullcoming* decision nonetheless left open whether the prosecution could introduce an analyst's testimonial forensic report (or transmit its substance) through an *expert* witness. The Court granted certiorari in *Williams* to answer that question, electing to review the Illinois Supreme Court's holding that the prosecution may introduce testimonial statements in the forensic reports through expert witnesses because statements introduced to show the basis for an expert opinion are not introduced for the truth of the matter (Hearsay Rule).

This conclusion is the most important aspect of *Williams*. Before the Court's decision, numerous state and federal courts had held that the prosecution could introduce testimonial statements not only through forensic experts, but also through mental health experts, "gang experts," and other experts.

So where, in practical terms, does this leave us? In the realm of forensic evidence, the Confrontation Clause continues to deem *formal* forensic reports testimonial. That means that drug, blood, alcohol, fingerprint, ballistics, autopsies, and related reports that typically involve testing by one person and that are incriminating on their face will continue to be inadmissible without the testimony of their authors (or some other method of satisfying the Confrontation Clause). Don't be tempted to get "tricky" with the rule. Justice Thomas in a footnote stated that "informal statements" are also testimonial when made to "evade the formalize process" previously used to generate such statements. It is time for the forensic expert to get an education in courtroom survival.

References:

1. *Williams v. Illinois*, 132 S.Ct. 610 (2012)
2. *Crawford v. Washington*, 124 S.Ct. 1354 (2004)
3. *Melendez – Diaz v. Massachusetts*, 129 S.Ct. 2527 (2009)
4. *Bullcoming v. New Mexico*, 131 S.Ct. 2705 (2011)

Forensic Testimony, Expert Testimony, Forensic Expert

E16 Judges' Evaluation of Evidence When Considering Post-Conviction Relief

Kayla A. Burd, BA, and Dawn McQuiston, PhD, Ariz State Univ, 4701 W Thunderbird Rd, Glendale, AZ 85306*

After attending this presentation, attendees will understand how recent U.S. Supreme Court decisions have impacted forensic expert testimony.

This presentation will impact the forensic science community by explaining how recent U.S. Supreme Court decisions concerning forensic experts and the Confrontation Clause directly affect their participation in the criminal justice system.

Any convicted person can file a petition to secure relief, which can include the granting of a new trial or resentencing for the offense. One may be eligible for post-conviction relief if: (1) the conviction violated the U.S. (or state) Constitution; (2) the Court was without jurisdiction to declare the judgment and/or sentencing it rendered; (3) the person's sentence exceeded the maximum allowed by law; (4) a person is held beyond his/her sentence; (5) a defendant failed to file a petition in a timely manner not due to his or her fault; (6) significant changes have occurred in the law that apply to the case; (7) the defendant can demonstrate clear and convincing evidence that the facts of the case are such that no reasonable fact-finder would have found him/her guilty beyond a reasonable doubt; or, (8) newly discovered evidence is found that, if presented at the time of trial, would be verdict-changing. This research examines the last of these criteria by evaluating judges' impressions of newly-discovered evidence and how these impressions impact a petition for relief.

In a post-conviction relief proceeding, a defendant can raise an argument concerning ineffective counsel or can outline a claim that evidence which existed at the time of trial but was only discovered *after* trial would in fact be verdict-changing in the minds of the jurors had it been presented originally. Petitions are initially reviewed by a Superior Court "gate keeper" judge who determines whether the petition is timely and whether a legitimate claim exists under the law. If these requirements are satisfied, the petition is then passed on to the original trial court Judge who determines if the claims necessitate a new evidentiary hearing. If a hearing is granted, the Judge could decide to grant a new trial.

This research aims to discover which evidence is most probative to judges when deciding whether or not to grant evidentiary hearings based on post-conviction relief petitions. The goal was specifically interested in learning about judges' impressions of evidence that reflects changes which have evolved in both science (e.g., DNA typing) and scientific theory (e.g., criteria for diagnosing shaken baby syndrome). Post-conviction petitions used in this research will be modeled after actual petitions filed in the state of Arizona. Judges will be asked to read a post-conviction petition which contains one or more of these types of evidence, and then will be asked questions about their impressions of the evidence presented and how likely they would be to grant an evidentiary hearing. It is predicted that certain changes in science, evidence quality, type of crime, and subjective beliefs will all play a part in the decision-making process when a Judge is appealed to during the post-conviction process.

This research and presentation will impact the forensic and judicial community by giving insight into judges' judgments and decision-making processes during the review of post-conviction relief petitions, and

determining which types of evidence (forensic or other) are most probative when considering these petitions.

Post-Conviction, Decision-Making, Evidence Evaluation

E17 How Many Expert Witnesses Does the Prosecution Need to Admit a Lab Report?

Lisa Kreeger-Norman, JD, 1613 Boyle St, Alexandria, VA 22314*

After attending this presentation, attendees will better understand the facts and holdings of the four Supreme Court cases which prescribe the manner in which the prosecution can admit lab reports into evidence and the number (and role) of the expert witnesses they must call to the witness stand. Expert witnesses who attend will learn "the rules" created by the four cases and will have an understanding of those rules. This understanding will enable forensic expert witnesses to know what to expect to hear about taking the stand from the prosecutor or defense attorney. The attendees will leave the session with an understanding of how the rules fit their discipline as, for example, sometimes DNA is different.

This presentation will impact the forensic science community by giving the necessary tools to prepare for the likelihood – or know there is little likelihood – that they will have to testify about the results reported in their lab report. This presentation bridges a gap in knowledge about what the U.S. Supreme Court has said in all of the opinions that address expert witness testimony that have been issued in 2004, 2009, 2011, and 2012.

Four cases which seemingly address the same Constitutional issue cannot be assimilated without looking at them as a whole. The Sixth Amendment Confrontation Clause guarantees the accused's right to confront the witnesses used against him. In 2004, the Supreme Court held, in *Crawford v. Washington,*[1] that the clause prohibits the Government from introducing out of court statements without putting the declarant on the witness stand. In 2009, in *Melendez-Diaz v. Massachusetts,*[2] the Court reaffirmed the decision, holding that forensic reports that certify results are testimonial statements. In *Bullcoming v. New Mexico,*[3] the Court held that when the prosecution introduces a forensic report, it must call the actual author of the report to the stand, not a supervisor or surrogate witness. *Bullcoming* left open the question of whether prosecutors can introduce an analyst's testimonial forensic report through a testifying expert witness. When can one testifying expert witness be used to admit another's forensic reports? Did *Williams V. Illinois,*[4] issued on June 18, 2012, answer the question?

Williams was issued as a plurality opinion: Judge Alito and three Justices wrote for the plurality, Judge Kagan and three Justices wrote for the dissent and one Justice seemingly ruled for both sides. Ultimately, Judge Thomas, writing alone, affirmed Sandy William's conviction for rape. How will the lower courts interpret *Williams* – narrowly or expansively? The answer is most likely they will do both. Does it depend upon whether it is a jury trial or a judge hearing the case alone? Does it depend upon the discipline the forensic science and author belong to? The final, integrated rule is that reports that are the internal work product leading up to a formal report are not testimonial and therefore not subject to confrontation. Will forensic scientists respond by re-categorizing the procedures that culminate in a final opinion? The *Williams* decision, combined with others, came with several warnings to prosecutors and forensic scientists regarding the number (and role) of expert witnesses that need to be called to the stand to introduce one report.

All four Supreme Court cases which address the issue of the Confrontation Clause as it applies to forensic expert witnesses will be discussed. There will be discussion about the application of the rules to different disciplines and different circumstances. Both forensic scientists and attorneys can, and should, tailor their expectations along the lines of these Supreme Court rules.

References:

1. *Crawford v. Washington*, 541 U.S. 36, 124 S.Ct. 1354, 158 L.Ed.2d 177 (2004)
2. *Melendez-Diaz v. Massachusetts*, 557 U.S. ____, 129 S.Ct. 2527, 174 L.Ed.2d 314 (2009)
3. *Bullcoming v. New Mexico*, 564 ____, 131 S.Ct. 2705, 180 L.Ed.2d 610 (2011)
4. *Williams v. Illinois*, 567 U.S. ____. Op 10-8505 (June 18, 2012)

Expert Witness, Laboratory Reports, Constitutional Law

E18 Designer Drugs: Legal and Analytical Challenges for Forensic Laboratories and the Courts

Heather L. Harris, JD, 2401 Lombard St, Ste 1, Philadelphia, PA 19146; and Barry K. Logan, PhD*, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

The goal of this presentation is to describe the evolution of the designer drug market in the United States, and describe the challenges laboratories and the courts are facing in detecting, identifying, quantifying, classifying, and reporting the latest round of synthetic drugs available on the street.

At the conclusion of this presentation, attendees will be able to: list the major classes of designer drugs and recognize something about their chemical differences; describe the limitations of current drug analysis techniques in identifying novel compounds; evaluate some of the approaches used by drug chemists in categorizing drugs; and, plan approaches to the prosecution or defense of drug possession cases based on accurate chemical information.

This presentation will impact the forensic science community by making attorneys and triers of fact more aware of the basis for the reliable forensic identification of novel drugs.

Over the past fifty years, a relatively small group of drugs including marijuana, cocaine, amphetamines, heroin, and a handful of diverted prescription opiates and sedatives made up the vast majority of illicit drug possession cases. The compounds were chemically distinct and diverse, well-characterized and well within the competencies of most forensic laboratories to detect and identify. Novel compounds appeared infrequently, for example MDMA or ecstasy in the late 1970's, and achieved low levels of adoption by the drug using community.

Starting in 2009, entrepreneurial chemists began clandestinely synthesizing drugs developed in the pharmaceutical industry as potential therapeutic agents, but with significant abuse potential, and introduced them first to the European, and in 2010 to the U.S., recreational drug markets.

The most popular of these drugs were synthetic cannabinoid agonists, drugs that bound to the cannabinoid receptors in the brain and produced marijuana-like effects. They included compounds like JWH-018 and HU-210. Around the same time drugs with stimulant and hallucinogenic properties that had been described in patents and publications in the 1980's began to appear, including methylone ("meow, meow"), and naphyrone ("NRG-1").

Following a series of deaths and intoxications resulting in injuries, state and federal legislators in the U.S. moved to schedule these compounds, but in a haphazard way resulting in a patchwork of inconsistent and difficult to interpret, laws and amendments to drug schedules. Drug manufacturers quickly moved to make new compounds whose scheduling status was less clear and possibly evaded the new laws.

The result was a mushrooming in the number of drugs now on the market. Initially dozens, and now hundreds of new chemical entities, are sold on the street, and in more mainstream outlets like smoke shops, convenience and novelty stores. Although the federal government in mid-2012 passed more comprehensive legislation to control these nationally, within weeks new classes of drugs began to emerge.

The compounds present challenges to forensic laboratories first in detecting and identifying the presence of novel compounds that may not be in their libraries or databases, confirming and quantifying drugs for which commercially available standards often don't exist, determining whether they are controlled substances or not, and determining whether they are reportable under the rules of their jurisdictions.

Specific challenges in the analysis and litigation of these cases arise from the fact that traditional analytical methods may not differentiate closely related compounds and even the latest techniques such as time-of-flight (TOF) mass spectrometry which are invaluable in providing molecular formula information, don't differentiate between isomers. Additionally, there has been insufficient time for the field to develop consensus around the interpretation of both legacy and the latest drug analog laws, which give either vague or very complex definitions.

The presentation will include some specific examples of emerging recreational drugs and assess their status as controlled substances or analogs to illustrate the challenges faced by forensic laboratories and the courts.

Designer Drugs, Controlled Drugs, Analog Laws

E19 The Independence of Medical Examiners and Forensic Pathologists — Redux

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After attending this presentation, attendees will understand some of the pressures placed on forensic pathologists and medical examiners and how that may affect the ability of defense attorneys to appropriately understand and possibly counter claims of state witnesses.

This presentation will impact the forensic science community by describing the outcome of a case in Minnesota and the response of that state's Supreme Court, addressing the issue of independence of medical examiners.

In 2008, a county attorney in Minnesota sent a threatening email to that county's medical examiner, stating that neither he nor the Sheriff would support her appointment as the county medical examiner if she or others in her office consulted with or testified for defense attorneys. The county attorney was subsequently publically reprimanded by the Minnesota Supreme Court for "engaging in conduct that is prejudicial to the administration of justice."[1] The trial that led to his sending the email was in a different and unaffiliated Minnesota county and the outcome resulted in a young woman being found guilty of pre-meditated murder for the stabbing death of her newborn baby. She was 17-years-old at the time of her baby's death, and was sentenced to life imprisonment without the possibility of release.

After a post-conviction hearing held before the trial judge, postconviction relief was denied. The case was then appealed directly to the Minnesota Supreme Court. The court exercised its supervisory powers and reversed the conviction in the interests of justice, due to the conduct of third-party state actors which undermined "the defendant's constitutional rights, contravened clear legislative intent, and interfered with the statutorily-mandated independence of medical examiners."[2]

The decision of the Supreme Court included clear guidelines regarding the role of medical examiners, stating "It is not a conflict of interest for a medical examiner to consult with criminal defense attorneys or testify at a criminal defendant's request. Indeed, such activity is authorized and protected by law."[3] They also found that "It should be undisputed that the quality of forensic investigation improves when medical examiners operate free from the influence of law enforcement and prosecutors."[4] Particularly heartening was the affirmation that "It is not disloyal for a medical examiner, who may testify as a State expert in the future, to consult with defense counsel or testify as a defense witness. Good medical examiners do not choose sides."[5] In conclusion, the opinion stated, "If Minnesota's law enforcement and prosecutorial communities believe that medical examiners are not independent, autonomous, and neutral actors, we now state clearly that such a belief has no place within Minnesota's criminal justice system."[6]

It remains to be seen if this ruling will have a practical effect on the access of criminal defense attorneys in Minnesota to local forensic pathologists. Many forensic pathologists are reluctant to consult with or testify for defense attorneys. Reasons include the unfortunate competition in Minnesota between medical examiner offices for counties and the perception that county attorneys in the counties being pursued prefer forensic pathologists who do not work with defense attorneys. Additionally, there are few forensic pathologists in the state and concern has been expressed that disagreeing with one another may lead to tension and conflict within this mostly close-knit group. Financial issues, including the perception that a forensic pathologist is "doing it for the money" discourage others from consulting.

This presentation seeks to share the experience in Minnesota and to continue the discussions between forensic pathologists and attorneys on how best to achieve just results in criminal proceedings.

References:

1. *State v. Beecroft*, 813 N.W.2d 814, 864 (Minn. 2012) (quoting, Minn. R. Prof. Conduct 8.4); see also, *In Re Backstrom*, 767 N.W.2d 453, 453 (Minn. 2009).
2. *State v. Beecroft*, 813 N.W.2d 814, 820 (Minn. 2012).
3. Id. at 832.
4. Id. at 833 (citing, Nat'l Research Council of the Nat'l Acads., *Strengthening Forensic Science in the United States: A Path Forward* 241 (National Academies Press 2009) available at http://www.ncjrs.gov/pdffiles1/nij/grants/228091.pdf.
5. *Beecroft*, 813 N.W.2d at 848.
6. Id. at FN 19.

Independence, Expert, Influence

E20 Not a Single Forensic Technique Has Been Examined for Validation or Scientifically Validated During the Four Years Since the National Academy of Sciences Report Issued: Are We Losing This Rare Opportunity to Bolster Credibility in Our Justice System?

Thomas L. Bohan, PhD, JD, MTC Forensics, 54 Pleasant Ave, Peaks Island, ME 04108*

After attending this presentation, attendees will learn of the forensic reform efforts over the past four years and of the conflicting forces displayed in that period that will shape the outcome of those efforts.

This presentation will impact the forensic science community by implementating without further delay the forensic-practices validation studies called for by the National Academy of Sciences recommendations.

In February 2009, the American people were gobsmacked by the National Academy of Sciences Report: *Strengthening Forensic Science in the United States: A Path Forward.* Gobsmacked, because they suddenly learned that their presumptions about the forensic techniques used to convict, incarcerate, and even execute their fellow citizens were misfounded. Whereas they, along with most judges in the country, had not doubted that those techniques were solidly supported by science, a committee of the body the country had relied on for scientific advice for more than a century now said unanimously that apart from forensic DNA not a single forensic practice of the nation's crime labs had ever been validated. Though the committee said much more, none of the rest drew the reaction elicited by the no-validation assertion, coming as it did in the midst of what seemed like weekly announcements of persons being exonerated long after conviction and imprisonment for heinous crimes.

The legal community's reaction was influenced by both the continuing exonerations and *Daubert* and *Kumho Tire.* Those two decisions of the U.S. Supreme Court require that expert-testimony reliability be established before that testimony can be introduced as evidence at trial. Clearly, one cannot establish the reliability of testimony based on a forensic practice that has never been found to be valid.

In contrast to the surprise of the public, the lawyers, and the judiciary, those scientists knowledgeable in forensics were not at all surprised, a circumstance that many news stories about the Report commented on with their own surprise. The scientists had always been

aware of the lack of validation of many forensic practices. They were also aware of the futility of trying to convince non-scientists of this through the adversarial system. For the forensic scientists, therefore, the February 2009 Report represented a long-sought opportunity to move outside the adversarial system to publicly examine the degree to which existing forensic practices have already been validated and, for those found by this examination *not* to have been validated, to measure scientifically the degree to which they *are* valid.

The forensic scientists' optimism in early 2009 was boosted by the swiftness with which the federal government, both the Executive and Legislative branches, reacted to the report (which had been produced in response to a request from Congress). Within months, a subcommittee on forensic science created by the White House and charged with advising the President on how to implement the report's recommendations was staffed and functioning. Concurrently, the Senate Judiciary Committee started holding hearings and meetings aimed at developing forensic reform legislation. It was at this point that conflicts emerged among the different "stakeholder" interests.

The White House subcommittee has had to keep its proceedings confidential until the President decides which of its recommendations are to be accepted. However, the Senate Judiciary Committee's work has been largely open and therefore provides a window into the struggle among those with very different concepts of what should take place, struggles that began just before the report was released and are continuing full throttle today. Those who attended the meetings called by the Judiciary Committee staff and/or attended the formal Committee hearings saw first-hand the lobbying being driven by the struggle. The outcome of the lobbying as of January 2011 can be seen in the form of the bill introduced that month by Senator Leahy, Chair of the Senate Judiciary Committee (the Forensic Reform Act of 2011), and as of July 2012 when a sharply revised version of that bill was released for comment. From the outset, one strong lobbying group has sought to have no important changes occur. A second group sees the reform movement as strictly a means of gaining more resources for crime lab directors. A third group, which may be the same as the first, is probably the strongest and has fought very hard to prevent one of the National Academy of Sciences fundamental recommendations from having any impact. Commenting on the failure of the Department of Justice (DOJ) to have corrected the long-existing failure in forensic practice, the National Academy of Sciences committee strongly expressed its recommendation that future forensic oversight be handled elsewhere than in the DOJ and indeed that the crime labs be removed from law enforcement agencies.

Given the political minefield in which they had to do their work, the Judiciary Committee staffers responsible for drafting the bill did a masterful job. The Forensic Reform Act of 2011 provided for an Office of Forensic Science housed in the office of the Deputy Attorney General (that is, within the DOJ), but for most control and staffing to reside in the National Institute of Technology and Standards (NIST), a purely scientific/technical agency of the federal government. Disappointingly, for those who wish to have scientific and not law-enforcement control of the federal oversight of forensic practice, the version of the bill released for comment 18 months later had been revised so as to effectively lodge control completely within the Department of Justice.

The same month that the revised Senate Judiciary bill was released for comment, a very different forensic-reform bill, the Forensic Science and Standards Act of 2012, was introduced concurrently in the Senate and in the House of Representatives. The new bill, developed by the Senate Commerce Committee, has a narrower focus than the earlier one. Whereas the bill developed by the Judiciary Committee seeks to address most of the recommendations of the National Academy of Sciences Report, the Commerce Committee bill is focused on the validity of forensic practices. In further contrast, the new bill assigns responsibility for the validation work to the National Academy of Sciences, NIST, and the National Science Foundation, with just a minor role for the Department of Justice. Reportedly, this bill was developed with little or no input from the group seeking to minimize change, the group seeking to increase resource allocation to lab directors, and the group that wished to retain in law enforcement the various responsibilities that the National Academy of Sciences Report recommended to be removed from that quarter.

As of August 1, 2012, the hope engendered by the National Academy of Sciences Report that forensic practices were on the verge of examination in non-adversarial setting has been completely unfulfilled. As indicated above, there are many intractable reasons for this failure. One not mentioned is the bundling by both the White House subcommittee and the Senate Judiciary bill of the validation studies into a myriad of other proposed fixes estimated to cost hundreds of millions of dollars and to require a large administrative structure. Both groups have lost sight of the fact that the public was upset because of the validation issue. Its demand was for *that* issue to be dealt with; all the matters that these two groups have spent most of their time dealing with pale into insignificance with that issue. What a disaster it will be if the omnibus solutions fail because of the various parties opposing one or another of their parts and, failing, drag down with them the them the validation work, the validation work which can be completed at a fraction of the cost and a fraction of the time, and would be by far the most important step in establishing the credibility of the nation's crime labs and dedicated forensic practitioners and hence of the US system of justice.

NAS Report, Validation, Poitical Pressures

E21 Ensuring Justice: What Lawyers and Judges Can Do to Carry Out the Goals of the NAS Report

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After attending this presentation, attendees will understand what tools are available to lawyers and judges to fulfill their role in carrying out the goals of the National Academy of Sciences (NAS) Report and ensure that only reliable forensic evidence is used in the court system.

This presentation will impact the forensic science community by exploring and examining what tools exist within the court system and how these tools should be utilized in the effort to carry out the goals of the NAS report. This is beneficial because it will help gain a better understanding of the interplay between forensic science and the courtroom.

The NAS Report entitled, *"Strengthening Forensic Science in the United States: A Path Forward"* was a call to action. Specifically, it was a call to action by the forensic science community. While it did not directly address the trial court's role in making the changes called for by the Report, this does not mean that the entire justice system, including both the trial court and counsel, is off the hook. In fact, because forensic science plays such an important role in so many cases before the court, it is imperative that the justice system take on its own role in implementing the goals of the NAS Report and ensure that justice is being served in our courts.

To ensure that justice is being served in our courts, judges and lawyers must start looking more critically at forensic evidence. As lawyers and judges we need to make sure that the forensic evidence being presented to juries is reliable. In order to determine whether the evidence is reliable, first and foremost, courts must ensure that the evidence being presented is generally accepted by the scientific community. In addition, lawyers and judges need to look at what processes were in place when testing is done. The presentation explores the lawyer role in the need to ensure the proper protocols are followed when testing is done. The judicial systems needs to ensure the proper safeguards are in place when testing is done and that the individuals conducting the testing are qualified. In order to make these determinations, those in the judicial system need to be able to examine the processes, protocols and safeguards.

In this presentation, the tools lawyers and judges have to ensure that only reliable forensic evidence is presented in court to either juries or judges will be presented. Generally, the judiciary looks to *Frye* in some state courts or to *Daubert*, as codified in Rule 702 of the Federal Rules of Evidence, in other state courts and federal court to determine whether the evidence being presented is reliable. This presentation will

examine whether these are the appropriate standards and whether these standards are being applied appropriately? Under *Frye*, the Court is to determine if the evidence being proffered is generally accepted in the general scientific community. If *Frye* is the standard, what additional tools exist to ensure that the procedures and protocols being used are sufficient? Are there other tools within the rules of evidence that should be used to carry out the goals of the NAS Report and ensure that only reliable evidence is being presented? Additionally, this presentation will explore whether lawyers should be challenging the reliability of forensic evidence on a global level or on a case by case basis? Finally, who bears the responsibility of ensuring that only reliable forensic evidence is introduced in court to judges and juries?

This presentation will benefit the forensic science community by exploring and examining what tools exist within the court system and how these tools should be utilized in the effort to carry out the goals of the NAS Report. This is beneficial because it will help us gain a better understanding of the interplay between forensic science and the courtroom.

Reliable Evidence, Frye, Daubert

E22 Forensic Nurse Collaboration With the Legal Team

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The goals of this presentation are to: (1) explore the implications of the Institute of Medicine (IOM) report for the future of forensic nursing specific to practice, education, and research; (2) explain how nurses can be full partners, collaborating with physicians and other forensic science professionals, in pursuing robust prevention pursuits to improve safety; (3) examine the commitment to moving the science forward; and, (4) outline advanced educational and training requirements for forensic nurses. The objective of this presentation is to outline the expertise of the advanced practice forensic nurse in collaborative roles within the legal system.

This presentation will impact the forensic science community by providing a basis for legal professionals to understand the expertise of advanced practice forensics within the legal system.

Regardless of the preparation level of the nurse, the goal of forensic education is to provide nurses who are knowledgeable in the care of all victims and perpetrators of violence and who are prepared to work in collaboration with professionals in the legal system. The advance practice forensic nurse has an educational background in psychiatric assessment and intervention skills, death investigation, forensic wound identification, evidence collection, family violence, sexual assault of all types and in varied populations, introductory law, and principles of criminal justice and forensic science.

Advanced forensic education for nurses prepares them to function in roles not held by nurses before. This is an emerging area of nursing practice and one that is in demand due to the increased incidence of violence within society, in general, and in the health care system, specifically. Among the roles of the advanced practice forensic nurse are that of the forensic nurse expert in the hospital or community setting who provides expertise across disciplines, risk manager, death investigator, nurse coroner, legal nurse consultant, death row mitigator, forensic psychiatric nurse, disaster nurse expert, corrections nurse, among others. These roles prepare nurses to provide expertise as nurse specialists in the courtroom as well as to serve as fact witnesses.

It is only with this advanced education that new roles emerge. In 2010, the Robert Wood Johnson Foundation (RWJF) and the Institute of Medicine (IOM) released an historic report, "*The Future of Nursing: leading change, advancing health."* It was felt that improving health and healthcare for Americans could not be done without the nursing profession. The focused outcome was to be an initiative regarding the Future of Nursing. The committee developed four key messages:

- Nurses should practice to the full extent of their education and training.
- Nurses should achieve higher levels of education and training through an improved education system that promotes seamless academic progression.
- Nurses should be full partners, with physicians and other health professionals, in redesigning health care in the United States.
- Effective workforce planning and policy making require better data collection and an improved information infrastructure.

It is this advanced education that prepares forensic nurses to collaborate with professionals within the legal system. Forensic nurses function as experts, fact witnesses, consultants, death row mitigators, nurse lawyers, and in general, serve to connect the health care community with the legal system in serving both victims and perpetrators.

Collaboration, Forensic Nurse, Roles

E23 What Lawyers Need to Know About Forensic Art

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After attending this presentation, attendees will understand: (1) the fundamental aspects of forensic art and its part in a criminal investigation; (2) what the prosecution and defense need to know, the qualifications of professional Forensic Artists (FAs); and, (3) the value for detectives in identification will also be covered.

This presentation will impact the forensic science community by raising awareness of the forensic art discipline and understanding how this discipline is an advantage when dealing with forensic drawings in the courtroom.

Forensic art has been used in the United States of America since the 1800s. The first and most common usages were for "wanted posters" of unknown criminals. Diagrams of crime scenes were also important aspects to documenting criminal behavior. Forensic art is an expanding field that is practiced throughout the United States as well as internationally. There are over 175 practicing FAs connected to law enforcement agencies across the country. Forensic artists produce ***information generating images*** that help investigators develop more leads and ultimately solve cases.

Computer facial imaging programs have had limited and disappointing results. Most operators are focused on how to use the software, rather than on their witness and conducting a neutral and productive interview. Few of these operators have the skills to render a lifelike image, and software training is limited.

Composites usually illustrate a suspect's image but may also be of a witness, or another victim that is sought. An illustration of a tattoo, stolen items, or sometimes vehicles are also described to a forensic artist for the creation of an image. The discipline also includes other, equally complex, applications. Principle areas include facial reconstruction, postmortem drawings, age progressions, digital imaging, and approximation drawings from videotape. With each application, the detective is relying upon the professional assistance of a forensic artist to get more information about a person involved in their investigation.

Both prosecution and defense need to know the same things. Is the artist employed by or connected with a law enforcement agency? How the drawing was developed and was it done without prejudice? Was the forensic artist trained specifically in forensic art? Training is key and the cognitive interview, an essential part of that. Composites are an illustration of what the witness recalls. This interview process assists the artist with witnesses who are traumatized by the event, gripped by the fear of retaliation for cooperating with authorities or lying about what they saw.

Tools and methodologies can vary around the country. Many forensic artists use a facial identification reference book, take notes and sign their work while others do not. There is a wide latitude in the construction of the drawing but focused agreement on the narrow

constraints involved for not leading the witness and neutrality in the interview. Certifications are available but not often required and there is no national certification at this time.

In some cases, forensic artwork may outright illicit an identification, in other cases it often plays a supporting role of corroboration or elimination. A jury reacts to a drawing that shows similarity to the defendant, knowing that the victim or witness was sincere in their description. They like to understand the process that developed that image.

Forensic art is a discipline that requires specialized training, above and beyond advanced drawing skills. Applications relate closely with those of other disciplines of the forensic sciences, especially forensic anthropology, forensic odontology and forensic psychology.

Forensic Art, Face Reconstruction, Forensic ID

E24 Introduction to the Science Behind Trace Evidence Examination

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After attending this presentation, attendees will gain a general introduction to the scientific method employed in the analysis of trace evidence, as well as the conclusions that can be expected from the various types of trace evidence examinations. Evidence included in the trace evidence category, such as animal and human hairs, natural and synthetic fibers, condom lubricants, gunshot residue, and paint will be discussed along with the different analysis methods used to examine those evidence types. Regarding the analysis methods used to examine trace evidence, a general but informative overview will be given on several analytical methods including optical and Polarized Light Microscopy (PLM), Scanning Electron Microscopy–Energy Dispersive Spectroscopy (SEM-EDS), Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, and Gas Chromatography – Mass Spectroscopy (GC-MS). The objective of discussing the analytical techniques is to expose the attendees to general types of evidence each method would be appropriate for, as well as what results that can be expected from each.

This presentation will impact the forensic science community by explaining the analytical methods used for analysis of trace evidence, along with the importance in the process of finding, interpreting, and properly testifying about the evidence. Even though trace evidence is usually circumstantial, and often cannot be used to identify an individual, it still plays an important role in forensic science. In addition to the direct comparison of questioned and known samples, trace evidence is vital in the evaluation of physical evidence to provide investigative leads to investigators in a variety of criminal investigations.

Trace evidence may be left behind at the crime scene, found on victims, and also taken with the perpetrators of crimes. The exchange of these traces often occurs without the knowledge of the perpetrator and is sometimes crucial in establishing a connection between the crime scene, the victim, and the suspect. DNA examination has evolved into a significant type of forensic evidence; however, trace evidence may be particularly important in establishing these connections in situations where suspect DNA is not recovered from a victim or the perpetrator's DNA profile is not listed in databases which law enforcement would use to make an identification of an unknown suspect. To give a better understanding of the occurrence of trace evidence, the authors will discuss the Locard Exchange Principle and different methods of transfer which can result in the depositing of various types of trace evidence.

This presentation will also discuss the strengths and weaknesses of the different trace evidence examinations, as well as what conclusions can be reached and how forensic scientists reach their conclusions and utilize trace evidence. As trace evidence is most commonly used as associative evidence as opposed to evidence that yields an identification of a suspect, the realistic strength of trace evidence analysis conclusions will be discussed, as well as some effective questions that can be asked in the courtroom to best portray to the jury the gravity of the results. Several case studies will be presented in which trace evidence played a crucial role in investigations and trials. Trace evidence is a valuable tool for forensic investigations as well a providing a solid scientific foundation to the trier of fact when determining the possible associations present in forensic casework.

Trace Evidence, Scientific Method, Evidence Transfer

E25 Effective Use of an Expert in a Premises Liability Case

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After attending this presentation, attendees will: (1) learn the elements involved in a premises liability case; (2) understand how a forensic engineering science expert can help evaluate the merits of a slip/trip/miss and fall incident; and, (3) be able to participate in preparing the case.

This presentation will impact the forensic science community by informing those involved in civil litigation related to slip/trip/miss and fall incidents, discussing the strengths and pitfalls of actual cases.

When pedestrians unexpectedly encounter a sudden change in a walkway surface, they might not adjust their gait sufficiently to prevent loss of balance and avoid a fall. The sudden stop of their body at the end of the fall may result in injuries severe enough to merit a claim for damages. Slip/trip/miss and fall accidents as premises liability cases are usually subjects of civil litigation.

Slip and fall cases usually involve a sudden decrease in available friction between the walkway and footwear contact surfaces. The unsuspecting pedestrian typically loses traction on the bottom of the leading foot when it meets the slipperier surface. The leading foot slides forward out from under his/her body and he/she falls onto or toward the side of the leading foot. Injuries to the trailing leg and/or knee are also possible. Less common is slippage of the trailing foot typically resulting in a fall onto the knee or leg of the trailing foot.

Trip and fall cases usually involve a sudden increase in elevation along the walkway. The unsuspecting pedestrian typically fails to lift the leading foot high enough to clear the transition to the elevated surface and instead catches it on the rise or edge of the transition. The leading foot does not support the pedestrian and he/she falls forward onto or toward the side of the leading foot. Less common is tripping when encountering a sudden increase in surface friction resulting in the leading foot not sliding as in preceding paces.

Miss and fall cases usually involve a sudden decrease in elevation along the walkway. The unsuspecting pedestrian typically fails to extend the leading foot downward far enough to meet the lower surface, stumbles, and falls forward onto or toward the side of the leading foot.

The forensic engineering science expert investigation typically begins with evaluating a description of how and where the pedestrian fall occurred. Appropriate hypotheses and alternatives for the slip/trip/miss and fall incident are developed. The incident site is evaluated for surface variations or irregularities that are consistent with the hypotheses and may have contributed to the incident. Determinations are made as to whether sudden changes in walkway surface existed at the time of the incident. If so, then evaluations are made to establish whether they are related to design, construction, or maintenance. Reviews of codes, standards, and statutes are made for applicability and for possible violations relative to the incident. Design and construction defects probably have statutory time limits for recovery of damages. (e.g., Minnesota has a limit of 10 years for design and construction defect claims.)

Indoor incident sites are usually subject to building codes and standards. Interior walkway features addressed by them include, but are not necessarily limited to, stairways, landings, ramps, handrails, guard rails, doors, dimensions, and illumination. Occasionally, design and construction features will be encountered that are not explicitly addressed in the written codes and standards and broad interpretations are evaluated for applicability.

Most exterior incident sites are not directly covered by building codes. The codes might be used as a guide for good practice but not be legally enforceable.

Exterior walkways are usually exposed to conditions of rain water and winter snow and ice. Thus, weather data for the hours and days immediately preceding the incident are often significant when evaluating causation. Drainage from overhead roof structures and along walkway surfaces can be a significant factor. Surface debris, color pattern, and illumination can also be significant factors.

A slip/trip/miss and fall incident involves contact or lack thereof between the walkway surface and the footwear contact surface. Thus, the footwear should be evaluated for possible contribution to causation.

Pedestrian conduct might be a contributing factor. If so, then the question of whether or not that conduct is reasonably foreseeable to an alleged negligent party must be addressed.

Pedestrian, Falls, Walkways

E26 A Criminal Case Can Turn on a Maggot

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After attending this presentation, attendees will understand the importance of collecting forensic entomological evidence in criminal investigations, its impact to cases due to the lack of the forensic entomological evidence, and how to ensure that forensic entomological evidence is collected.

This presentation will impact the forensic science community by providing case examples of the importance of collecting insect evidence by law enforcement officers for use in prosecuting criminal offenses.

The prosecution of criminal cases involves proving the various elements of criminal statutes. Law enforcement officers are the initial responders to criminal activity and are responsible for the amount and quality of evidence that is collected from the crime scene. In some jurisdictions, prosecuting attorneys are regularly consulted by the law enforcement officers during the processing of the crime scene. This consultation can facilitate the proper collection of necessary evidence, the legality of evidentiary issues, and certain additional avenues of investigation that need to be pursued.

Unfortunately, when communication between law enforcement officers and prosecuting attorneys is lacking, crucial evidence can potentially be lost, thus causing future proof issues at trial. This is especially true with forensic entomological evidence. Forensic entomological evidence, in the form of blow fly eggs, larvae, and adults, is usually only found and available for collection at the initial crime scene and/or autopsy. Insect evidence can provide law enforcement officers and prosecuting attorneys valuable information on time of death, location of death, length of insect infestation, and in some cases identification of the perpetrator. If police officers fail to collect this insect evidence during the first response to the crime scene, the evidence is unlikely to be present days, weeks, or months later. Without this entomological evidence, important elements of the crime may not be established, such as time or location of death in murder cases or time of insect infestation in abuse cases.

There are several reasons why the necessary entomological evidence is not collected. First, individuals in law enforcement may not be aware of the importance of insect evidence and lack the knowledge regarding proper collection techniques for the preservation of specimens. In addition, law enforcement officers and crime scene technicians may not want to take the additional time necessary to collect the “bug” evidence or wait for a forensic entomologist to arrive to assist them in recovery. Finally, those collecting the evidence may feel that the entomological evidence is not important because they have other evidence that will determine the time frame.

In order to ensure proper collection of forensic entomological evidence, police, as well as coroners and medical examiners, need to be informed of the importance of collecting this evidence. Prosecuting attorneys need to be familiar with the information that this evidence can provide for their cases. By being familiar with the answers that can be provided by forensic entomology, law enforcement and prosecuting attorneys can ensure that these answers are not lost by failure to collect and retain the necessary evidence.

Prosecuting attorneys need to be familiar with the elements of a crime that entomological evidence can determine and be able to communicate with law enforcement officers, coroners, and medical examiners to make sure that this important evidence is not lost. Proper collection of essential evidence ensures that the guilty are convicted and the innocent are exonerated.

Criminal Justice, Forensic Entomology, Evidence Collection

E27 Search Warrant Language for Cloud Computing

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After attending this presentation, attendees will be able to understand and create search warrants for data in cloud-computing environments. Attendees also will be able to enumerate potential challenges facing the production of cloud-based evidence.

This presentation will impact the forensic science community by arming the legal and digital forensic communities with language for cloud-specific search warrants, an example warrant based on a case study, and a list of potentially relevant cloud-based forensic artifacts.

Cases involving evidence from cloud computing environments will soon dominate criminal and civil litigation and the legal community must be armed with the knowledge about how to execute e-discovery of cloud evidence. Companies are embracing cloud technology to offload some of the cost, upkeep, and growth of equipment that they would otherwise have purchased themselves. The general public is increasingly storing their data in clouds, sometimes unknowingly. Cloud infrastructure – with exceptional bandwidth, storage, and computing power – offers an attractive prize for hackers. Where the people, the data, and the money go, so too does crime. While many people have lamented how the users of the cloud and their data are protected, few of these discussions have considered the difficulty of responding to security breaches, including forensics and criminal prosecution.

This presentation gives the first public discussion of search warrant language that could be used to compel cloud-based data from a cloud provider. Law enforcement and legal professionals may be unfamiliar with this new technology, and therefore unsure what electronic evidence may exist and what to seize in a warrant. Many audience members will be familiar with e-discovery of online webmail and social media, but cloud computing differs in important ways that make acquisition of data different. This presentation will rely on technical expertise of cloud computing to suggest possible language for use in a search warrant. In particular, friendly definitions of technical cloud terms, and a list of potentially relevant data to ask for in a warrant will be presented. This work builds on discussions with cloud providers about the forensic data they collect, and incorporates prior work from legal scholars about the legal challenges associated with cloud-based data.

A hypothetical case study of child pornography being hosted in the cloud will be used to illustrate suggested wording and phrases to use in a search warrant. While fictional, it describes a common computer crime where the cloud is an accessory to a crime. This sample language is useful for agents and prosecutors who wish to obtain a warrant authorizing the search and seizure of data from cloud computing environments. In particular, this example is tailored to Amazon Web Services, the largest and most popular public cloud service provider.

Following the discussion of warrant language, several potential shortcomings of cloud-based e-discovery in general will be enumerated. The defense could use the deficiencies identified to challenge and discredit the process and product of cloud-based evidence. Such issues include the untrustworthiness of cloud data, extreme complexity of the cloud environment, and likely failure of the *Daubert* test.

Now is an exciting time for cloud computing as innovative new product offerings emerge. The legal community is also at the threshold of a wave of cloud-related litigation. The first public cases involving cloud-based ESI are likely to appear soon, and the people involved in

those cases have a unique opportunity to set new legal precedent. This exploration of seizing electronic evidence from cloud computing provides a foundation to forensic investigators and legal professionals as they investigate and prosecute cloud-based crimes.

Cloud Computing, Digital Forensics, E-Discovery

E28 Visualization of Mobile Evidence: Timelines, Maps, and Social Graphs

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After attending this presentation, attendees will understand the advantages of using software to visualize forensics data and "see" patterns and relationships in the digital contents of mobile devices. In doing so, data is elevated into information, or even knowledge, that attorneys can use with their clients for decision making, for strategy formulation, and for taking meaningful action in a legal, investigative, or corporate context. The presentation will include technical information on software bridges that span leading mobile forensic examination tools and popular timeline, mapping, and investigative software packages used by attorneys and investigators today. Evidence visualizations created by this technology will be briefly demonstrated for the audience.

This presentation will impact the forensic community by proposing new evidence visualization techniques available to both criminal justice attorneys and civil litigators that have the potential to contribute greatly to just legal outcomes in and out of the courtroom. Many trials today, whether in federal or state court, involve showings of complex factual information produced as digital evidence. But how can justice be served if the trier of fact cannot understand the evidence and the story it can tell? Therefore, it is imperative that complex digital evidence be presented in such a way as to be relatively straightforward for the judge or jury to understand. The visualization of digital evidence is one technique to provide that understanding cost effectively.

Digital evidence is usually presented today as narrative and numbers accompanied by a stream of bits and bytes in computer forensic reports that are at best sorted into buckets of similar items. For example, mobile phone evidence is presented such that call logs appear in one section split into inbound, outbound, and missed calls. Text messages appear elsewhere split into inbound and outbound messages. The phone's address book appears yet somewhere else. This primitive and fragmented approach at production hides or makes opaque the evidence story that attorneys desperately need to develop a theory of the case for presentation to juries to win at trial. Technically, forensics data is made available to attorneys and their clients, but often this evidence is so difficult to interpret and understand that they derive little, if any, value from it. Why? Because many digital forensic examiners report their findings one-dimensionally and out of context. Unfortunately, legal professionals, who are not technologists, cannot relate to it. They miss the substance and the importance of the evidence's meaning and its ability to tell the story of the case.

The hypothesis or proposition of this presentation is that forensic information must be visual in order to be optimally useful and valuable to attorneys and the clients they serve. It puts forward the idea of using software to visualize forensics data and "see" patterns and relationships in the digital contents of mobile devices. In so doing data is elevated into information, or even knowledge, that attorneys can use with their clients for decision making, for strategy formulation, and for taking meaningful action in a legal, investigative, or corporate context. Attorneys, investigators, forensic accountants, and others most effectively perform their work with timelines and maps, not data streams. They map and chart factual information like events, actions, incidents, messages, calls, and so forth.

This presentation will show how digital forensic examiners can put meaningful evidence to use with attorneys, opposing counsel, judges, juries, and their own legal teams. The focus is on how meaning can be mined from forensics data cost effectively using software automation. It will include technical information on software bridges that span leading mobile forensic examination tools and popular timeline, mapping, and investigative software packages used by attorneys and investigators today. Evidence visualizations created by this technology will be briefly demonstrated for the audience.

Furthermore, visualization capabilities have as much evidentiary value earlier in the legal process as they do at trial in the courtroom. Depositions, motion practice, and pre-trial hearings present excellent opportunities for sharing and communicating the meaning of forensic data. So do settlement conferences. Why? Today so few cases actually go to trial. The legal community knows that less than 10% of cases at the federal level are tried. Many are subject to decision by plea bargains, settlement conferences, arbitration, and even mediation. Yes, the majority of cases today are settled on or before the courthouse steps. Modern evidence visualization techniques can tell the story of the case and contribute great value to justice in and out of the courtroom.

Visualization, Mobile, Evidence

E29 Comparison of the Current Italian Legislation Regarding Assisted Reproduction With the Other European Union Countries: What's New?

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After attending this presentation, attendees will learn about a comparative legislative analysis regarding assisted reproduction in European Union (EU) countries.

This presentation will impact the forensic science community by discussing important regulatory and legal differences among EU nations in the provision of assisted reproduction, taking into account peculiar aspects concerning the handling of embryos, use of donors, surrogates, and embryo research.

This report reviews the 2012 legislation concerning Assisted Reproduction (AR) in 27 EU Member States, trying to compare the Italian legislation with the other European countries. The collection of data was completed on January 31, 2012.

In 2004, a broad range of legislative quality and safety requirements for the donation, procurement, testing, processing, preservation, storage, and distribution of tissues and cells was introduced by the European Parliament and the Council with the launch of the Directive 2004/23/EC1. Taking into account the wide coverage of the Directive in comparison to the very specific nature of AR treatments, all EU Member States except Belgium have implemented the EU Tissue and Cells Directive.

Nineteen EU Member States (FI, SE, UK, DK, NL, DE, BE, FR ES, PT, IT, AT, SI, CZ, SK, HU, BG, GR, EE) have reported the existence of AR specific legislation in their own countries, whereas 8 countries (CY, IE, LT, LU, MT, PL, RO, LV) have reported not having AR specific legislation but general legislation covering AR procedures. For Romania, although no AR specific legislation is in place, there exists a general law based on Tissues and Cells Directive for all kinds of cell and tissue transplants (Law n° 95/2006 and law guidelines/25.10.2006 with all later amendments) which covers AR treatments.

In Italy, in May 2009, the Italian Constitutional Court modified the law regulating assisted reproduction technology (ART) which had been approved in February 2004 by the Italian Parliament, eliminating most of the limitations. The 2004 law had led to considerable reproductive migration as it imposed so many limitations on infertile Italian couples: e.g. no more than three oocytes could be fertilized at any one time during IVF or ICSI treatment; all embryos had to be transferred into the uterus; it was forbidden to freeze embryos or screen them for genetic or

chromosomal successful pregnancy and oocyte and sperm donations were prohibited. The application of the Law was regulated by governmental guidelines.

In recent years, in EU Countries, ethical debates about the scope of medical services available to enhance a couple's ability to procreate have resulted in laws that curb access to certain procedures. These laws, derive from different origins ranging from an extremely prohibitive approach in IT, DE, and AT, versus a cautious regulatory approach in DK, SE and FR and the liberal regulatory approach in the UK, ES, and NL.

There are important regulatory and legal differences among EU Nations in the provision of AR services, including divergence on key issues such as the handling of embryos, use of donors and surrogates, embryo research, and PGD treatment.

Assisted Reproduction Law, Italian Law N° 40/04, Ethics and Law

E30 The Scientific Question vs. the Legal Burden: How Much DNA Testing Is Enough?

Ted R. Hunt, JD, Jackson County Courthouse, 415 E 12th St, Fl 7M, Kansas City, MO 64106*

After attending this presentation, attendees will better understand the differences between the scientific question posed to DNA analysts and the legal burden faced by prosecuting attorneys, as these distinct concepts relate to different levels of proof required in forensic science and law.

This presentation will impact the forensic community by enhancing interdisciplinary understanding of the important distinction between these two concepts. This presentation will also facilitate a greater appreciation of the necessarily dynamic, rather than static nature of the "scientific question" posed to analysts by prosecutors who must respond to a moving target—an ever-evolving theory of defense—while attempting to satisfy the legal burden of proving the defendant's guilt beyond a reasonable doubt.

Typically, the "scientific question" is initially posed to a DNA analyst by law enforcement. Answering that question consists of determining whether, to a reasonable degree of scientific certainty, a known biological standard can be associated with questioned crime scene stain—and if so, what amount of weight that conclusion deserves. In many cases, the scientific question is asked and answered before an arrest is made and provides the basis for the prosecutor's decision to file charges.

However, an incriminating answer to this evidence specific question does not conclusively establish the prosecution's theory. Rather, it supports an inference in favor of that theory. Accordingly, analysts must remember that a single, ostensibly strong DNA association to a criminal defendant is not necessarily sufficient to satisfy the prosecutor's burden of proof in court. As the case proceeds toward trial and the defense begins to take shape, the original scientific question may evolve, may be reframed, or it may be necessary to answer altogether new scientific questions. Thus, additional DNA analysis may be necessary to shore up a case for prosecution, to rebut a defense attack, or respond to newly-developed facts and/or fluctuating legal theories.

From the analyst's perspective, a prosecutor's request for supplemental DNA analysis of one or more additional *probative* evidence stains may seem pointless, redundant, and unnecessary. This belief may stem from the analyst's lack of intimate familiarity with the factual context of the originally tested evidence, and/or the litigants shifting legal issues and theories relative to that evidence. It may also arise from the analyst's unfamiliarity with the prosecutor's task—qualitatively different than answering the scientific question—of proving the case beyond a reasonable doubt to a unanimous 12 member jury. The potentially different perspectives of prosecutors and analysts regarding the need for supplemental DNA testing highlight the distinction between the "scientific question" and the "legal burden."

The "legal burden" shouldered by American prosecutors is to prove each element of a criminal offense beyond a reasonable doubt. The prosecutor's theory is informed by the credible facts in the case and the admissible incriminating evidence. In many cases, the single most incriminating fact may be the DNA test results offered by the prosecution's expert witness.

However, it must be remembered that the defendant also has a theory of the case. Furthermore, the defense is never legally obligated to disclose or declare its theory to the prosecution. That theory may ultimately be that the prosecutor has failed to meet his or her burden of proof, may incorporate and utilize different and additional facts and evidence, or may call for a contrary and competing interpretation of the same facts.

The ability of the defense to constantly update its theory in response to the government's case results from legally recognized distinctions between the parties. Unlike the prosecution, the defense has no burden of proof; must *never* declare his/her trial strategy at any point in time; is entitled to categorical acquisition, review, and use of the prosecution's discovery; and may largely ignore the same discovery rules and deadlines that the prosecutor is obligated to follow. In some cases, the result will be a defense that resembles a moving target—constantly evolving and updating in reaction to the prosecution's case. In this way, the defense will attempt to accommodate, avoid, or refute the prosecution's DNA evidence with whatever strategy counsel deems most plausible at *any* given point in time.

Because of this legal asymmetry between the parties, corroborative (sometimes called convergent) evidence is essential to any prosecution. Corroborative evidence may be defined as that which is supplementary to evidence already given and that tends to strengthen or confirm it.[1] In those cases in which DNA provides the sole or primary link between a suspect and a crime, supplemental DNA analysis of one or more available *probative* evidence samples may provide essential corroboration for the prosecution's existing DNA evidence. Such testing can also simultaneously diffuse defense attacks against the contextual significance of the evidence, its chain of custody, the analyst's skills and experience, the lab's evidence handling procedures, testing protocols, and the interpretation of the results. In sum, targeted supplemental DNA analysis can serve to greatly diminish the courtroom plausibility of various defense attacks against a single incriminating DNA result, while simultaneously increasing the chances of a just and successful prosecution.

It is recognized that laboratories may fear that the prosecution's need for corroborative DNA evidence will present a number of practical and logistical challenges. Among these are unpredictable rush jobs, disrupted work flow, increased pressure placed on already limited resources, and greater risk of error due to artificially imposed deadlines. These fears, however, should not cause labs to refuse to test supplemental *probative* samples that may corroborate existing incriminating DNA results.

One solution to this problem – either wholly absent or extremely rare in many jurisdictions is the consistent interdisciplinary communication and collaboration between prosecutors, law enforcement, and laboratory officials. Forensic case coordination, or "triage" meetings, should take place between representatives of these agencies before or immediately after criminal charges are filed in cases whose success will heavily depend upon DNA testing and analysis. The main purpose of these interdisciplinary meetings should be to collectively discuss the facts of the case, potential legal issues, various possible theories of the prosecution and defense, and to make informed decisions about which *probative* items of evidence should be tested. Such meetings also provide a forum for prosecutors and lab officials to share their potentially different perspectives on the need for supplemental DNA testing, and allow them to justify and defend their positions at a point in time in which such testing remains a realistic possibility.

In addition, follow-up interdisciplinary case meetings or communications should take place as needed. During these meetings, the prosecutor should update laboratory officials on the current status of legal issues and evolving theories of both the prosecution and defense. Participating officials should also collectively decide whether any additional remaining *probative* items of evidence should be analyzed to further strengthen the prosecution's case, or to rebut a potential attack

from an unfolding defense theory. In this way, the "scientific question" will remain relevant by frequent updating and refinement based on the current legal and factual exigencies of each case.

Reference:

1. Black's Law Dictionary 182 (5th ed. 1983).

Scientific Question, Legal Burden, DNA Analysis

E31 DNA in the Courtroom: Top Ten Tips From the Bench

Arlene Perkkio, JD, Dakota Co District Court, 1560 Highway 55, Hastings, MN 55033*

After attending this presentation, attendees will understand ten concrete practicing tips on how to present DNA in a courtroom. The tips are drawn from in court experiences of a state court trial judge.

This presentation will impact the forensic science community by explaining how scientific evidence is a growing issue in court cases and showing the importance for lawyers and judges to explain DNA evidence in a user-friendly language.

Attendees will understand ten concrete practicing tips to assist in improving understanding and communication with others of the importance and value of DNA evidence. These practicing tips will focus predominately on how to present DNA evidence in a courtroom. The ten concrete practicing tips are drawn from actual in court experiences of a state court trial judge.

This presentation will impact the forensic science community by providing an explanation from the perspective of a state court trial judge, of how scientific evidence is a growing issue in court cases and courtrooms across the country. This presentation will also impact the forensic science community by showing why it is important for lawyers, judges and scientists to explain DNA evidence in simple, easy, understandable, user-friendly language.

Judges serve as the "gatekeeper" to the admissibility of forensic science evidence. Yet many lack scientific training and must rely upon the lawyers coming before them to explain how DNA evidence is or is not relevant and should or should not be admissible. The central issues addressed in this presentation are: how do lawyers, with little or no scientific background, explain DNA evidence to other lawyers and judges, with little or no scientific background? How do lawyers and judges insure jurors, with little or no scientific background, understand DNA evidence? How do scientists insure lawyers, judges and jurors understand scientific evidence?

Understanding and then explaining scientific evidence is a growing issue in court cases and courtrooms throughout the country. Attorneys and judges have a tendency to scan over scientific evidence, getting to the end result, the findings, and then disregarding the underlying data. This presentation will show the importance of understanding the DNA evidence, its significance as related to the facts in the instant case, and common sense application of this information. If the analysis says the DNA matches, most attorneys look no further; this presentation will stress why this should be a starting point, the beginning, not the end of the analysis.

Lawyers who are able to effectively understand and then break down scientific issues into understandable language get their desired results. Lawyers, who can explain the science in simple and understandable language, understand the science. The ten concrete practicing tips will: (1) focus on what lawyers and judges can do to educate themselves about the science of DNA; (2) demonstrate how to explain the forensic scientific information; and, (3) will explain what scientists can do to help lawyers, judges, and jurors understand DNA evidence.

Data from DNA court cases, compiled over an 18-month period, gathered by the author will be reviewed. This presentation seeks to offer practitioners, judges, and scientists information about best and worst practices in the courtroom for DNA court cases. Lawyers and judges have taken different approaches to understanding, handling, and presenting DNA evidence. This presentation will provide a composite which will educate the lawyers, judges, and scientists.

This presentation seeks to inform attorneys, both prosecution and defense, and scientists as to what should be considered when attacking the very real problem of addressing the "gatekeeper," the judge, about DNA evidence. The ten concrete practicing tips will focus on what scientists and lawyers can do to explain the science of DNA in court cases to judges with no science background.

DNA, Courtroom, Lawyer

E32 Preventing DNA Wrongful Convictions: Legal and Judicial Responsibilities

Brian C. Zubel, JD, PO Box 70, Fenton, MI 48430*

After attending this presentation, attendees will recognize that the bench and bar have important responsibilities to provide not only legal analysis in criminal cases, but also an independent review of forensic testing results and interpretations. By accepting reported results of forensic testing at face value, attorneys and judges run the risk of overlooking important exculpatory evidence, errors, and misinterpretations.

This presentation will impact the forensic science community by demonstrating how forensic DNA methods and their interpretation can be highly subjective. In order to prevent wrongful charges and convictions, both judges and attorneys must develop an independent capacity to scrutinize the conclusions claimed by laboratory personnel.

Forensic DNA results are susceptible to a wide range of interpretation, particularly where DNA mixtures are concerned. Studies such as NIST MIX05 have demonstrated that differences in the interpretational guidelines used by different laboratories can influence the conclusions claimed in DNA mixture cases. Even DNA analysts from the same lab and using the same interpretational guidelines have reached very different results when examining the exact same data. Given the range of inter- and intra-laboratory interpretational variation, it falls upon the legal profession to provide additional tiers of meaningful review.

In 2009, a homeowner returned to find his house burglarized and a number of firearms stolen from his gun cabinet. The homeowner saw that the cabinet had been pried open and that one of his kitchen knives, the blade bent into a curve, was lying at the foot of the cabinet. Police investigators collected the knife as evidence; both it and a swabbing taken from the handle were submitted for forensic DNA typing.

Employing AmpFℓSTR® Profiler Plus® and COfiler® amplification kits, the laboratory obtained a male profile across the thirteen CODIS DNA loci. Additional alleles were also detected, but these fell below the laboratory's validated reporting threshold of 150 rfu. Lacking any suspect at that time, the unknown male profile was entered into CODIS.

More than a year later, police investigators arrested a suspect they found in possession of firearms which had been stolen in other burglaries in the area. They obtained a search warrant for buccal swabs and submitted these reference samples to the laboratory for comparison. As it turned out, the suspect's buccal swabs were processed by a different DNA analyst than the one who had processed the knife handle swab.

The second analyst never processed any evidence obtained from the knife; relying entirely on the data generated by his colleague a year earlier. Looking at the exact same electropherograms, the second analyst reached an entirely different conclusion. Instead of agreeing with the first analyst that the knife handle represented DNA from an unknown male, the second analyst concluded it was instead a mixture of types from at least two individuals ***and that the suspect could not be excluded as a possible contributor to the mixture***.

These two conclusions are obviously irreconcilable. The first analyst had correctly reported the unknown male profile to CODIS. When the suspect's known profile is compared to the CODIS profile, the suspect is ***excluded*** at eight of the thirteen DNA loci tested. The second analyst had improperly concluded that the sample was a mixture, taking into consideration allelic activity that was clearly below the laboratory's validated interpretational threshold.

The government laboratory in question employed internal procedures for the administrative review of reports, yet the obvious error

in this case was not spotted. Nor was it detected by the police investigator who submitted the matter to the prosecutor for a warrant, or by the prosecutor who issued charges. Fortunately for the suspect, the defense team was able to bring the mistake to the prosecutor's attention. All charges were dismissed on the day scheduled for preliminary examination.

While this case might constitute anecdotal evidence of "analyst bias," it also provides a sobering reminder of how easily the criminal justice system can fail. If DNA wrongful convictions are to be prevented, lawyers and judges must have the technical ability to scrutinize claims made by forensic scientists.

DNA, Wrongful Convictions, Mixed Samples

E33 Death Row Defense: Investigating and Evaluating Forensic Issues in Post-Conviction

Matilde Carbia, JD, 3050 Grand Rt St John, New Orleans, LA 70119; and Gary Eldredge, JD, 205 S Scott St, New Orleans, LA 70119*

After attending this presentation, attendees will understand the role that guilt and mitigation investigations, and the forensic issues commonly developed through such investigations, play in the representation of death row inmates in the state post-conviction stage of appeals. The importance of post-conviction investigation in preventing the execution of wrongfully convicted defendants will be underscored, as will intellectually disabled defendants who are constitutionally barred from receiving the death penalty.

This presentation will impact the forensic science community by educating attendees about the variety of forensic issues encountered in death penalty defense, and the ways in which a legal team identifies and develops these issues in post-conviction. The forensic community will also gain insight into how the work of capital defense organizations and innocence projects have proven that wrongful convictions are far too common to be acceptable in our criminal justice system.

Over 130 people have been exonerated from death rows around the United States since 1973.[1] Between 1973 and 1999 there were an average of three exonerations per year.[2] From 2000 through 2011 there have been an average of five exonerations per year.[3] In a 2001 study that looked at the cases of eighty-six wrongfully convicted death row inmates dating back to 1973, the three leading causes of wrongful convictions were faulty eyewitness identifications, government misconduct, and the testimony of jailhouse informants.[4] However, mishandled or unpreserved evidence or the use of unqualified experts was a contributing factor in almost ten percent of the wrongful convictions.[5]

In post-conviction, capital defense organizations systematically review client's cases for evidence of innocence, prosecutorial misconduct, and other indicia of wrongful conviction. Representing capital clients in post-conviction requires that an attorney become knowledgeable in multiple forensic disciplines in order to spot potential forensic issues, successfully develop them through investigation and expert involvement, and cogently present them to a judge in the form of legal claims. During the guilt phase and mitigation phase investigations that take place in post-conviction, forensic issues are frequently encountered and must be fully explored.

A guilt investigation focuses on the development of forensic issues related to the crime and the physical evidence, while a mitigation investigation focuses on trauma and mental illness in a client's family history.

Several steps of a guilt investigation involve the forensic sciences:

- Careful preliminary review of the prosecution's forensic evidence, including lab reports, the crime scene, and lay and expert testimony;
- Finding, examining, and evaluating the physical and scientific evidence collected and developed during pre-trial law enforcement investigation; and
- Consulting with experts and considering independent defense scientific testing and analysis.

Steps in a mitigation investigation that involve the forensic sciences include:

- Making preliminary assessments of a client and his family members for potential mental health or intellectual functioning issues; and
- Consulting with forensic mental health experts to fully develop a history of mental illness and/or brain damage that was not presented at trial.

Hypothetical case examples will be presented in order to illustrate a sampling of issues that are frequently encountered in the course of guilt and mitigation investigations in capital post-conviction.

References:

1. Staff Report, House Judiciary Subcommittee on Civil & Constitutional Rights, Oct. 1993, with updates from Death Penalty Information Center (www.deathpenaltyinfo.org).
2. *Id.*
3. *Id.*
4. Warden R. How mistaken and perjured eyewitness identification put 46 innocent Americans on death row: an analysis of wrongful convictions since restoration of the death penalty following *Furman v. Georgia*. Berrien Springs (MI): Andrews University, 2001.
5. *Id.*

Capital Defense, Fact Investigation, Wrongful Conviction

E34 Girls Rule, Perps Drool: How an 84 Pound Teenager Made Her Attacker Bleed All Over the Evidence

Melissa Mourges, JD, and Martha Bashford, JD*, NY County DA Office, One Hogan Place, New York, NY 10013*

After attending this presentation, attendees will understand how to use a "John Doe" indictment to preserve jurisdiction against the statute of limitations, focusing on an attempted murder/sexual assault case in which a teenage girl fought her attacker so fiercely she drew blood, but the perpetrator's forensic profile had no match in CODIS.

This presentation will impact the forensic science community by providing an insight into what kinds of cases make good candidates for "John Doe" indictments and how to proceed when a match finally occurs in CODIS.

It was just two days after her 15th birthday when Jessica Reyes, measuring 4′8″ and weighing only 84 lbs, entered the lobby of her building in a huge housing complex in upper Manhattan. She got into an elevator, intent on her cell phone, and as she was about to exit, she was grabbed from behind by a man who put her in a stranglehold and threw her onto the floor. He choked her to unconsciousness and pulled her off the elevator on the top floor, 34 stories above the ground. Surveillance video captured this blitz attack.

When Jessica came to, he had her in the stairwell, alternately choking and punching her in the face. Hoping to avoid something worse, Jessica gave him her only possessions, a student subway metrocard and a single dollar bill. He threw her to the floor and they tumbled down three flights of stairs, fighting and clawing at each other. The assailant tore off her pants and underpants. When he took his hands off her to pull down his zipper, she fled to her own apartment. She was bleeding profusely, with blackened eyes, a torn lip, a broken nose and bruises all over her body.

The stairwell looked like a charnel house. Crime scene photos show clots and smears of blood where Jessica's head repeatedly bounced off the stairs. Crime scene investigators recovered a pair of men's eyeglasses and the blood-stained metrocard from the stairwell. DNA analysis developed the same male profile from each item and the profile was uploaded to CODIS. No match was found.

Fearing that the statute of limitations would run out, in 2007 the Cold Case Unit of the Manhattan District Attorney's Office obtained a

"John Doe" indictment. "John Doe with a Particular DNA Profile" was charged with attempted murder, attempted rape and other crimes to stop the clock. Because New York is a grand jury state, that means the victim had to testify in person, knowing her assailant may never be caught.

Finally, a CODIS "hit" matching to a man named Steven Carter. As a result of a conviction for a home invasion against an elderly man with Parkinson's disease, Carter's DNA entered CODIS on September 10, 2010. The match occurred on September 13, 2010. It took CODIS three days to solve a crime that had lingered unsolved for almost seven years. Carter pleaded guilty on the eve of trial. Jessica Reyes became the public face of the power of DNA as she crisscrossed New York state encouraging legislators to pass an "All Crimes" DNA Databank expansion law, requiring offenders convicted of all misdemeanors and felonies to go into CODIS. Her efforts paid off; that law was signed into effect this past May.

Sex Crimes, John Doe Indictment, CODIS

E35 Collecting DNA From Arrestees: An Examination of Policies, Practices, and Implications

Julie Samuels, MPP, Elizabeth H. Davies, BS, and Dwight B.N. Pope, BA*, Urban Institute, 2100 M St NW, Washington, DC 20037*

After attending this presentation, attendees will understand the variations in these laws and how key provisions in laws (e.g., the scope of the qualifying offenses for which samples are collected, the point in a case at which samples are obtained or analyzed, the policy for expunging DNA profile records) have been translated into practice, all of which impact the operations and infrastructures of state crime laboratories and law enforcement agencies. Special attention will be paid to the workload implications of these provisions for state crime laboratories and law enforcement agencies, as well as the impact these provisions have on CODIS.

This presentation will impact the forensic science community by highlighting implementation lessons generated from the experiences of other states and helping states understand the implications of such an expansion on agencies responsible for implementation. Further, the presentation will not only address how such laws impact CODIS, but also how criminal justice stakeholders can begin to measure the effectiveness of collecting DNA at arrest or charging.

As of July 2012, more than half the states and the federal government have enacted laws authorizing DNA collection not only from individuals who are convicted, but also from individuals who are arrested for or charged with certain qualifying offenses. The Urban Institute's Justice Policy Center, with support from the National Institute of Justice has studied the investigative utility of these laws and how they impact the law enforcement agencies and state crime laboratories responsible for their implementation. The study team conducted a thorough review of relevant state laws, interviewed state crime laboratory representatives and various key stakeholders, and collected summary laboratory workload and CODIS data.

While the scope of qualifying offenses varies across states, about half the states collect for all felony offenses. The rest of the states authorize collection from a subset of felony or misdemeanor offenses. The scope of collection not only impacts the volume of samples crime laboratories receive and subsequently analyze (e.g., laboratories, for instance, may need to hire additional personnel), but it also impacts the administrative workload of both law enforcement agencies responsible for collection and laboratories. Further, while laws authorizing the collection of DNA prior to conviction have been referred to as "arrestee" DNA laws, the point at which collection occurs varies widely across states. Most states that have such laws collect DNA as part of the booking procedure. However, others have set additional requirements for collection. In some states collection is not legally authorized until an individual has been formally charged or arraigned, or a judicial determination of probable cause has been issued. Last, the process for initiating the removal – or expungement – of DNA profiles from CODIS upon acquittal or case dismissal varies among states. In general, few laws require the states to automatically carry out an expungement if an individual is eligible, while most laws require that the individual initiate the process.

Because of case attrition from arrest to conviction, the collection of DNA *prior* to conviction typically means that law enforcement is drawing from a larger universe of individuals than if collection only occurred upon conviction. Indeed, based on data collected from states, the total number of samples laboratories have received since implementation typically surpass the volume of samples received annually prior to implementation. However, this growth may be limited in states that bear the burden of expunging profiles. While the number of matches – or hits – between known and forensic profiles have increased over the years as the number of profiles have increased, the attribution of the growth in hits to the laws' expansion is challenging to substantiate.

DNA, CODIS, Arrestee

E36 Low Copy Number Y-STR Evidence: Will "Junk" DNA Dilute the Power of the Phrase "DNA Match" and Debase the "Gold Standard?"

Scott M. Kozicki, JD, Law Office of Cook Co Public Defender, 69 W Washington, Chicago, IL 60602*

After attending this presentation, attendees will become aware of the emerging issues surrounding low copy number Y-STR evidence in criminal trials and the concerns of prosecuting and defending these types of cases.

This presentation will impact the forensic science community by challenging participants to debate about the wisdom of prosecuting cases using low copy number Y-STR DNA evidence, and whether new guidelines are needed to maintain the integrity of forensic DNA technology.

The phrase "DNA match" carries terrific power. It derives from the notion that the DNA associated with a crime is essentially unique; it can virtually and statistically single out the accused as the source of the DNA evidence. What happens when laboratories and prosecutors begin calling "DNA matches" with one-in-four probabilities, when the DNA evidence might actually match three members of the jury? Will that "DNA match" claim sometimes be wrong, associating some accused individuals as included when they might be excluded? Will subjectivity and bias increase? Will powerful science be mixed with junk science? Will the meaning of the phrase "DNA match" become diluted?

These concerns are increasing in the forensic courtroom setting, especially in Y-marker cases. The Y-chromosome short tandem repeat (Y-STR) markers have been used in criminal investigations to help detect low levels of DNA where other kits have failed, targeting only the male contribution in the evidentiary sample. As DNA science and technology advances, forensic labs are now able to get test results from smaller quantities of DNA, even when these test results yield incomplete and partial DNA. In some instances, these Y-results are reported as "DNA matches" even when the profile is derived from only one loci detected. In fact, lab analysts have reported such test results, defended them in court, and have been supported by their management teams.

The use of low copy, Y-STR DNA results leads to differing mixture interpretations. Typically, Y-STR loci produce single alleles at each of the ten (out of the eleven loci available in the kit) loci in single source samples of male DNA. As a result, lab analysts interpret these Y-STR results as single source samples. When forensic analysts observe multiple peaks at any one of these ten loci, they commonly report that more than one male contributor is present in the tested sample. Absent from this forensic analysis is when a particularly challenging problem arises: while many individuals have only one allele at each of these ten loci on the Y-chromosome, some individuals have two or even three alleles at some loci. Accordingly, a laboratory report interpreting the Y-result as a mixture of at least two contributors and "matching" the defendant's DNA haplotype could likewise be interpreted as a DNA

exclusion. The use of this low copy, Y-STR DNA evidence at a criminal trial could ultimately result in the most egregious failure in the criminal justice system: the conviction of the innocent, while allowing perpetrators to walk free.

This presentation will show examples of this emerging issue from actual casework and trials. Attendees will learn about the challenges presented by this new issue, and be challenged to engage in a debate within the forensic science community about the wisdom of such practices, and whether new guidelines are needed to maintain the integrity of forensic DNA technology.

Y-STR, DNA, Mixtures

E37 Minnesota Trial Team: Public Defenses' Responses to State-Sponsored Forensic Science

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After attending this presentation, attendees will recognize that, within the national criminal justice system, the forensic education of lawyers in public defense is generally behind that of their counterparts in law enforcement and prosecution. Attendees will also recognize that because of this realized gap in forensic education, Minnesota's public defense system embarked on a rather novel approach in dealing with its unique lack of forensic knowledge and education of its public defense lawyers.

This presentation will impact the forensic science community by showing that the development of a concept, such as Minnesota's Trial Team, can significantly "level the playing field" for indigent criminal defendants when there is a state prosecution that contains significant forensic evidence generated by state-sponsored forensic laboratories. The forensic community will further understand that, with the creation of such entities, it enables the public defense lawyer to challenge the forensic scientist's testing methods, the results, the interpretations and, in some instances, even the very validity of certain forensic evidence being brought forth in today's criminal courts. The forensic community will ultimately understand that the criminal justice system is best served by both the state and defense having the ability to intelligently present, and question, significant forensic evidence presented at a trial.

In the years leading up to 2006, the Minnesota State Public Defender recognized that major felony trials in Minnesota were becoming increasingly involved with complex forensic issues and evidence. This was not only occurring within the major metropolitan area of Minneapolis and St. Paul, but more importantly in rural, Greater Minnesota. The Minnesota Public Defender system in greater Minnesota is manned by part-time personal that also have private practices. At the time, county attorneys representing small counties would routinely seek assistance from the Minnesota Attorney General's Criminal Division, and the accompanying forensic labs, for assistance in these complex, forensic cases. The part-time public defender did not have any like resource, nor experience, to draw upon concerning forensic trial issues.

In order to address this need, the State Public Defender created the "Trial Team." The Trial Team is comprised of five lawyers that have an over-all mission of providing high-quality, cost-effective assistance for trial representation in major felony cases that involved forensic evidence as well as to train other assistant public defenders in the understanding and use of forensic evidence. These lawyers are all trained in the science of DNA, and continue to update that training through the Minnesota Public Defender Advanced DNA Institute. They have also selected other forensic areas in which to become well versed in order to further their basic mission. These additional areas include arson, gunshot residue, psychiatry, pediatric forensics, fingerprints, ballistics, eyewitness identification, blood spatter and digital/electronic evidence.

With the advent of the National Academy of Sciences Report in 2009, the Trial Team's mission of statewide forensic education, support and assistance to local public defenders became more critical in order to adequately represent indigent clients. When requested by local co-counsel, this has meant that, with support of the Trial Team, local public defenders are now looking closer at the forensic evidence produced by local police and other state sponsored forensic laboratories on a pre-trial level. The Trial Team provides its specialized forensic knowledge, and litigation support as co-counsel, to local public defenders for the purpose of litigating those *Frye-Mack* challenges to questionable forensic evidence once considered above such challenges (fingerprints, handwriting analysis, ballistics, gunshot residue and shaken baby syndrome to name a few), to file other similar motions and to assist in the client's trial.

The National Academy of Sciences Report of 2009, the ongoing *Frye-Mack* challenges supported by the Trial Team and those public defenders trained or co-counseled by Trial Team members have led, in part, to the Minnesota Supreme Court advising the State District Courts that *Frye-Mack* challenges should be allowed in all cases with forensic evidence, even forensic evidence long-permitted by Minnesota Courts[1]

Reference:

1. *State v. Hull*, 788 N.W.2d 91 (Minn. 2010); *State v. Ferguson*, __ N.W.2d __ 2001 WL 4949998 (Minn. October 19, 2011).

Trial Team, Forensic Evidence, DNA

E38 Learning a Forensic Science Discipline Can Make You a Better Attorney

Julie A. Maxwell, JD, 510 15th St NW, Rochester, MN 55901*

After attending this presentation, attendees will understand how learning one aspect of forensic science contributes to increased proficiency in all areas of forensic science and improves trial skills for attorneys. The successes of participants in the Minnesota Public Defender Advanced DNA Institute will be used as examples of how investment in an in-depth forensic science program can increase forensic skills for attorneys. Successful incidents from actual cases will be described.

This presentation will impact the forensic science community by showing how in-depth DNA training for attorneys can improve litigation skills in this complex area. This training can also prompt attorneys to gain additional knowledge and skills in other forensic fields that can lead to better courtroom litigation skills.

The National Academy of Sciences (NAS) Report found that lawyers "often lack the scientific expertise necessary to comprehend and evaluate forensic evidence." In response, the Minnesota State Public Defender, working with a small group of attorneys, developed a year long training program to teach thirty assistant public defenders DNA typing litigation skills, using a combination of intensive lecture, small group discussion, and one-on-one tutoring. Each attorney applied this advance training to one of their own actual, pending cases. Prior to this training, a lot of attorneys just accepted the reports submitted by the forensic laboratory for their face value. Now, following the training, nothing is taken at face value. These lawyers increased confidence in their ability to spot issues and then to work with experts on complex forensic issues.

This presentation will explore what has occurred in the Minnesota Public Defender System since this program's training component was completed in 2011. Since this time, many of the attorneys originally involved in this program have demonstrated an increased interest in a variety of areas of forensic sciences. Using the structure put in place during the formal training period, lawyers have continued to collaborate with each other on cases using both a listserve and monthly group meetings. They have also returned to their own jurisdictions throughout Minnesota and worked with lawyers who were not involved in the Institute to improve the overall ability of lawyers throughout the public defender system to spot issues and litigate relevant subject matter. The ongoing work of these lawyers collectively as a group and individually, has led to the increased interest in other areas of forensics such as toxicology and digital forensics that impact on their practice of law.

Examples of cases that will be discussed include: challenges to the State's requests to obtain DNA samples from defendants before the known samples have been tested in criminal sexual conduct, assault,

and burglary cases, thus preserving clients' constitutional rights of privacy; dealing with a complex mixture in a criminal sexual conduct case; recovery of crucial evidence by retrieval of text messages from a broken cellphone which the expert has called "the smoking gun" evidence in a burglary and assault with a dangerous weapons case; and the probe into the policies and practices of the City of St. Paul Police Department Crime Lab.

This presentation will give a detailed explanation of how advanced training in forensics can lead to improved performance in the courtroom.

In-Depth Training, Improvement, Litigation Skills

E39 Investigating a Serial Killer: Jeffrey Dahmer, 20 Years Later

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After attending this presentation, attendees will gain an understanding of the specific roles of forensic pathologists, investigators, and criminalists investigating serial killers.

This presentation will impact the forensic science community by informing jurists and investigators on the contributions of forensic pathologists, investigators, law enforcement, and criminalists in the documentation, evaluation, and case preparation of a serial killer. By analyzing the the investigation of the Jeffrey Dahmer case, participants will experience the step-by-step sequence of scene, autopsy, and interview techniques, as well as the necessary collaborative environment and interactions required in a high profile death investigation.

On July 23, 1991, personnel from the Milwaukee County Medical Examiner Office and Milwaukee Police Department responded to an apartment where partially skeletonized remains of eleven individuals had been detected. Law enforcement had initiated the investigation of the premises after responding to a naked man, who while handcuffed, was running down a city street. The assailant, Jeffrey Dahmer, was quickly taken into custody. In this unique situation, Dahmer freely discussed his role in the killings with investigators and provided a valuable resource for the collaboration of the scene and autopsy findings to pathologists and law enforcement personal during the investigation. Medical examiner personnel recovered seven human skulls, three of which were painted; four human heads, and one postcranial skeleton from a portable freezer; and three skeletonized bodies from a 55-gallon plastic storage drum. The partially dissected bodies, skulls, and numerous photographic journals were transported to the medical examiner's office. Scene investigation, anthropological analysis, and autopsy details of the bodies provided prosecutors with valuable evidence of the methods of death, motives of the killer, and psychological state of Dahmer during the course of a death spree that lasted nine months.

The evaluation of the scene allowed investigators to establish methods, motives of death, begin the preliminary identification process, and to demonstrate the mental capacity of the assailant. Forensic pathologists assisted with the identification, established the cause of death, and documented injuries that allowed investigators to question Dahmer on various injuries he inflicted upon victims. Of the eleven victims recovered from the scene, all were identified on the basis of antemortem dental comparison by a forensic odontologist. The skeletons were easily identified due to characteristic dissection levels of the spine and extremities. Fingerprints were recovered from four victims. The anthropological and odontological examination assisted with the identification and also resulted in the establishment of victim profiles. Dahmer was diagnosed as having a mixed personality disorder with sadistic, obsessive, fetishistic, antisocial, necrophilic features typical of what has been described as organized, nonsocial, lust murderer.

This presentation will discuss investigation of the Jeffrey Dahmer Case from scene to the courtroom. It will discuss the physicial and pathological evidence used to convict Jeffrey Dahmer. It will also discuss the pitfalls in the investigative process using the lessons learned in the Dahmer case.

Serial Killer, Jeffrey Dahmer, High-Profile Case

E40 The DUI HGN Test: A Forensic Look at the NHTSA's HGN Robustness Study

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After attending this presentation, attendees will understand the importance of conducting a proper Horizontal Gaze Nystagmus (HGN) test in Driving Under the Influence (DUI) investigative cases.

This presentation will impact the forensic science community by reinforcing the necessity of conducting a proper DUI HGN test in the prescribed standardized manner to avoid false positive results.

The HGN test is a pre-arrest exam utilized by law enforcement in the United States of America. It is a component of a three battery testing procedure also including a nine step walk and turn test and a one leg stand test. These three components are collectively referred to as the "Standardized Field Sobriety Tests" or SFST.[1]

The SFST's are taught to law enforcement officers nationwide as a means to "standardize" the investigation of those motorists who are suspected of have having driven an automobile with an unlawful BAC.

The SFST's were developed in the Southern California Research Institute over 30 years ago with periodic updates published by the United States Department of Transportation's National Highway Traffic Safety Administration (NHTSA).[2]

The HGN test is described to law enforcement as "the involuntary jerking of the eyes" as they move from side to side and are further taught that if the HGN test is performed correctly revealing four or more clues that HGN is 77% accurate in predicting that a motorist's blood alcohol concentration is over 0.10%. A stimulus (often a pen or finger) is held between twelve to fifteen inches from the face at slightly above eye level.

The standardized procedures for the administration of the HGN test begins with screening the motorist for certain pathological disorders that include brain damage or diseases of the middle ear by looking for equal pupil size, resting nystagmus, and equal tracking (eyes following an object together).

The HGN test is then scored for a total of three clues per eye for a total of six clues.

The first component is checking the eyes for "the lack of smooth pursuit" by moving the stimulus two seconds out and two seconds back for each eye and then repeating the sequence to determine if the motorist's eyes are able to pursue the stimulus smoothly.

Next the motorists eyes are checked for "distinct and sustained nystagmus are maximum deviation" by holding the stimulus all the way across the motorist's face and holding that position a minimum of four seconds to observe the eye for distinct and sustained nystagmus. Both eyes are to be checked twice.

The third HGN test step requires the officer to check for "onset of nystagmus prior to forty-five degrees" by moving the stimulus at a speed of approximately four seconds to reach the edge of the motorist's shoulder. When HGN is observed the officer is told to stop and verify that the jerking continues. Again, both eyes are checked twice.

The NHTSA training manual emphasizes that the validation of the SFST's applies only when: "The tests are administered in the prescribed standardized manner."

A study of officers performing HGN tests on motorists being investigated for DUI revealed that in only one out of fifty two occurrences the HGN test was performed correctly.[3] The statistical evaluation of NHTSA's 1998 report "Validation of the Standardized Field Sobriety Test Battery at BAC's Below 0.10%" concluded that, "The accuracy is substantially less for individuals with lower alcohol levels."[4]

An improperly administrated HGN test creates a conflict between forensic science, law and public policy. When the HGN test has been compromised the government often endeavors to still justify the test results. Too often law enforcement or the prosecution attempts to minimize the flawed HGN test by extolling the statistics of the approved standardized test.

The NHTSA report, "*The Robustness of the Horizontal Gaze Nystagmus Test*" states that, "HGN as used by law enforcement is a robust procedure. The study findings provide no basis for concluding that the validity of the HGN is compromised by minor procedural variations."[5]

An evaluation of the "HGN Robustness Study" as applied to potential motorists .080% BAC or below will be presented. This evaluation reveals a high false positive result per the NHTSA SFST training received by police officers in relation to an arrest decision at 0.08% based on four or more ovserved HGN test clues.

References:

1. Tharp V, Burns M, and Moskowitz H., Development and Field Test of Psychophysical Tests for DWI Arrest. DOT-HS-805-864. National Technical Information Service, Washington, D.C., 1981
2. Burns M and Moskowitz H., Psychophysical Tests for DWI Arrest, DOT-HS- 802-424 National Technical Information Service, Washington, D.C., 1977
3. Booker JL, End-positional nystagmus as an indicator of ethanol intoxication, Science & Justice 2001: 41(2): 113-116
4. Hlastala M, Polissar N, Oberman S, Statistical Evaluation of Standardized Field Sobriety Tests, J Forensic Science, May 2005, Vol 50, No. 3
5. Burns M, The Robustness of the Horizontal Gaze Nystagmus Test. DTNH22-98-D-55079. National Technical Information Service, Washington, D.C., 2007

Alcohol, SFST, HGN

E41 EPA, GLP, and USP vs. Forensic Science: Where is the Commutability? Why Are There no Standardized Methods Across All of Forensic Science?

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After attending the presentation, attendees will appreciate the difference between the highly regulated world of analytical chemistry in the Good Laboratory Practices (GLP) and the Environmental Protection Agency (EPA) regulated environment, as well as USP guidelines versus the largely unregulated world in forensic science where there is no overarching oversight.

This presentation will impact the forensic science community by bringing into focus the discussion on the need for validated, standardized methods in forensic science with the prize not simply being proper metrology, but instead commutability.

Commutability is the hallmark of all measurement science and is particularly important in analytical chemistry at large. Commutability is the feature of being comparable across borders and universal over all time. It's goal is to set up a scheme so that a test of an unknown results in the same qualitative identification and a quantitative estimation regardless of where the testing happens (geographically and environmentally) and through all of time (this year, next year or 100 years later). Using appropriate and traceable standards/reference materials during calibration combined with using validated and standardized methods provides for commutability.

Since the mid 1980s, the Environmental Protection Agency (EPA) has pushed the forefront of standardizing assays and methods in testing then delivering validated and standardized methods for all laboratories to us throughout the United States and in the world. Shortly thereafter, the Food and Drug Administration (FDA) developed GLP whereby a method has to be proven as valid, standardized, and then is accepted and used throughout industry for a particular purpose. The United States Pharmacopeia (USP) has published a stringent set of guidelines that govern the validation and standardization of methods. These validation procedures include testing to determine:

1. Accuracy (Bias)
2. Precision (Calibration)
3. Specificity (Degree of Selectivity)
4. Limit of detection
5. Limit of quantitation
6. Linearity and range
7. Ruggedness
8. Robustness

Put basically and simply, a validated method means that there has been some sort of rigorous method of testing of the instructions of the assay and its calibration procedure to produce data that shows and proves that the method is suitable for its intended purpose.

A true validation study much like the ones found in GLP and EPA regulated environments are consistently missing in the world of forensics. Therefore, the true commutability of any measurement reported in the forensic world can be questioned. There is a high degree of variability in the world of forensics. Not so in GLP and EPA worlds.

Contrast this with the world of forensics. For example, blood ethanol analysis by way of Headspace Gas Chromatography using a Flame Ionization Detector (HS-GC-FID) is a routine method of analysis and perhaps the most frequently run forensic assay in the US today. Yet, unlike EPA, GLP, or USP methods, there are no standardized methods, instrumentation or assays developed and implemented in the testing world. Some laboratories conduct ethanol determinations by enzymatic assay, others use direct liquid injection on packed columns by way of GC-FID. Still others use single column GC-FID. One laboratory runs GC-FID with MS. Not all laboratories use dual column HS-GC-FID. Those that do run dual column HS-GC-FID some consciously decide not to quantitate on two columns. What constitutes a batch as well as what constitutes proper quality control is never uniform among laboratories. Acceptance criteria vary in a seemly arbitrary way. Should there be blanks between unknowns? Should inorganic salt be used? How much dilution should be performed by way of ISTD? And the list goes on and on showing a great amount of variance between and among laboratories to the point that the core question remains: where is the commutability of the number generated USP, VIM 3, and TAM sense of the word.

GLP, EPA, Commutability

E42 The Misleading Nature of Unconfirmed Single Column Headspace Gas Chromatography Flame Ionization Detection Blood Ethanol Reports

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After attending the presentation, attendees will appreciate the need for confirmation testing of forensic blood ethanol samples consistent with the published Society of Forensic Toxicologists and American Academy of Forensic Sciences (SOFT/AAFS) Forensic Toxicology Laboratory Guidelines – 2006 and the various gas chromatograph column manufacturer guidelines. The need for confirmatory testing will be evidenced by real life examples of unjust consequences that occur when these critical guidelines are not followed.

This presentation will impact the forensic science community by furthering the discussion that just because a blood ethanol report has the appearance of reliability does not unequivocally mean the results are an unquestionable true value.

For the past several decades forensic toxicology reporting of blood ethanol levels by crime laboratories have been relied upon by attorneys, juries, and judges largely without scrutiny to the qualification of the subject analyte, quantification of the reported blood ethanol level, or reporting of any uncertainty in the measurement. The 2006 SOFT/AAFS Guidelines dedicate a section to Confirmatory Tests. The Guidelines specifically provide that *"…confirmation using a second analytical system is encouraged…Confirmation using a second GC column is acceptable IF the second results in significant changes in retention time AND change in elution order of at least some of the common volatiles."* One major column manufacturer publishes, *"Don't forget: Blood alcohol analysis requires dual column confirmation when using GC/FID."* Yet many crime laboratories fail to adhere to either the 2006 Guidelines or the gas chromatograph column manufacturer's published warnings/instructions. Instead, these crime laboratories report blood ethanol results from only a single column.

The admissibility of blood ethanol reports and other forensic evidence have now been addressed in multiple recent United States Supreme Court decisions. These issues are now at the forefront of our country's high court and forensic community. Despite the guidance from the high court, state courts are slow and even reluctant to require a proper foundation prior to the introduction of blood ethanol reports which do not comply with SOFT/AAFS and gas chromatograph column manufacturer guidelines and warnings. State courts routinely allow unconfirmed blood ethanol results into evidence. Often times judges are persuaded to believe that the blood ethanol reports, inclusive of quantitation, should be published to the jury and admitted into evidence without being subjected to pretrial in-limine motions. Some state courts have ruled that the jury may determine what weight to give the blood ethanol reports without any substantial foundational showing. Judges are also faced with the challenge to determine what expert testimony regarding the blood ethanol report is required. These "weight versus admissibility" rulings appear more prevalently in states where the well known *Frye* "general acceptance" test is used and not the *Daubert* or federal standard which requires both a preliminary assessment of whether the methodology underlying the blood ethanol report is scientifically valid and whether that methodology properly can be applied.

Jurors rely upon the blood ethanol reports to convict defendants based on partial information, educated guesses, and the belief that the judge as the gate keeper would not allow the reports into evidence unless they were accurate, precise, and reliable.

Single Column GC, Unconfirmed Results, AAFS/SOFT Guidelines

E43 Legal Implications of Bitemark Evidence, Post-NAS Report: Is There a Problem?

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The goal of this presentation is to review the legal implications related to innocent people who have been shown to have been wrongfully convicted based upon erroneous bitemark identification evidence. Bitemark case false convictions have been a serious concern for the forensic science community and the criminal justice system for well over a decade. The educational objective of this presentation is to identify legal system errors and/or omissions made in bitemark cases were factual innocence has been demonstrated and propose solutions for improving the adjudication of cases were bitemark evidence is proffered. The attendee will understand the need for attorneys and trial judges to exercise caution in litigation of cases involving identity based in part or solely upon a bitemark comparison.

This presentation will impact the forensic science community by focusing on judges and attorneys who deal with cases involving bitemark experts to better understand issues related to bitemark evidence reliability.

DNA identity testing continues to exonerate innocent people who's cases involved bitemark comparison evidence. The problem of innocent people being convicted and unjustly imprisoned for crimes they did not commit became a significant concern to the forensic odontology community for the past decade and efforts to establish the science of a bitemark comparison have been and are being vigorously pursued. Two recent bitemark exoneration cases will be discussed. In a case from Wisconsin, Robert Lee Stinson, served 23 years of a life sentence for the 1984 murder of Lone Cychosz. Ms. Cychosz had been a neighbor of Mr. Stinson and her body was bitten a number of times in the course of a brutal assault that killed her. In 1986 Stinson's direct appeal of his first degree murder conviction was denied.[1] After DNA testing, the State of Wisconsin on February of 2008 agreed that Robert Lee Stinson should be granted a new trial. Mr. Stinson consistently maintained his innocence. In July of 2009, the State of Wisconsin dismissed the murder charge against Robert Lee Stinson. Thereafter in 2010, DNA evidence identified the actual killer of Ms. Cychosz. In a second case, Kennedy Brewer was accused by the State of Mississippi of the 1991 murder of Christine Jackson, the three-year-old daughter of his girlfriend. Kennedy Brewer was initially convicted of raping and strangling Jackson to death in 1995. He was sentenced to death and spent 12 years on Mississippi death row. In February of 2008, Kennedy Brewer's case was dismissed by the State of Mississippi after another person identified by DNA evidence confessed to killing the three-year-old girl. Both Stinson and Brewer were convicted due to bitemark evidence. In both the Robert Lee Stinson and Kennedy Brewer cases, the dental bitemark experts were board certified by the American Board of Forensic Odontology (ABFO). The Stinson and Brewer cases are examples of a number of cases where bitemark evidence has been shown to be erroneous. Many legal experts now claim that bitemark identification evidence should be held to be a junk science. Now, defense attorneys faced with bitemark evidence in a case routinely are filing legal challenges against the admission of bitemark evidence. The challenges contend that there is effectively no valid documented scientific data to support the hypothesis that bitemarks are demonstrably unique. Additionally, it is argued that there is no documented scientific data to support the hypothesis that a bitemark is a true and accurate reflection of this uniqueness. To the contrary, scientific evidence that does exist supports the conclusion that crime related bitemarks are grossly distorted, inaccurate, and therefore unreliable as a method of identification. A published study of the National Research Council entitled Strengthening Forensic Science in the United States: A Path Forward, in the specific section on forensic odontology, the NAS Study found that bitemark comparison was the most controversial area of forensic odontology and that there *"is continuing dispute over the value and scientific validity of comparing and identifying bitemarks."*[2] In its criticism of bitemark comparisons, the NAS Study stated:

> *"There is no science on the reproducibility of the different methods of analysis that lead to conclusions about the probability of a match." Even when using the American Board of Forensic Odontology guidelines, different experts provide widely differing results and a high percentage of false positive matches of bitemarks using controlled comparison studies."*

If bitemark evidence is to remain as viable evidence of identification in our judicial system specific measures must be taken to guard against any circumstance where a miscarriage of Justice could occur. One lesson learned from bitemark exoneration cases is that errors occur when an expert overstates the validity or certainty of a bitemark identification. Also, exoneration cases show the need to develop a minimum threshold of objective criteria for the suitability of a suspected bitemark before a comparison is ever attempted.

The investigation of bitemark cases by forensic dentists are evolving as the result of deficiencies uncovered after convictions which relied on bitemark evidence were overturned by DNA evidence. As a direct result of past bitemark case mistakes, there should be a better understanding by attorneys and judges within the legal system to recognize problems with bitemark evidence and put in place safeguards to protect against wrongful convictions. Courts should accept the reality that there is no scientific basis to allow a bitemark expert opinion that a person is a "positive match" to a suspected bitemark. The path forward: One approach would be to simply not allow bitemark evidence in any court case until a firm scientific basis is established. Another approach would be to limit the manner bitemark evidence could be used by a trier of fact in cases where a person is identified by a bitemark. For example, to assure that an innocent person is not wrongfully confined based upon bitemark evidence; a jury instruction could be tailored to limit the use of bitemark evidence. Specific jury instructions will be discussed.

References:

1. *State of Wisconsin v. Robert Lee Stinson*, 134 Wis. 2d 224; 397 N.W. 2d 136 (Wis. 1986).
2. The National of Academies Press, 2009 at p. 5-35 (hereinafter, NAS Study)

Bitemark, Wrongful Conviction, Reliability

E44 Bitemark Analysis: Foundation, Lessons From the Past, and Paradigm Shift to the Present and the Future

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After attending this presentation, attendees will better understand the scientific methodology of bitemark analysis, the evidentiary strengths of bitemark analysis, the roles of the bitemark analyst and bias, past cases of "bitemark analysis-gone-wrong," comments on the National Academy of Sciences Report, *"Strengthening Forensic Science in the United States: A Path Forward"* (2009), and the evolving future of bitemark analysis.

This presentation will impact the forensic science community by demonstrating the evidentiary value of the appropriate use of bitemark analysis. Despite a recent past that has included innocent persons to lose liberty and freedom as a result of expert testimony involving faulty bitemark analysis, there have been exponentially more cases where the use of bitemark analysis has resulted in protecting individuals and society from violent predators as well as interventions that have saved countless lives.

Bitemark analysis as a scientific investigation meets all the criteria for the definition of *science* with one notable exception, the ability to create and study bitemarks in living human skin. It is not currently possible to experimentally create and study biting patterns in living human skin. All other aspects of the application of scientific methodology in bitemark analysis are valid, accurate, and scientifically sound.

When a bitemark injury is considered to be of high evidentiary quality, the presence of semi-circular opposing arch forms depicting the maxillary and mandibular teeth, each with distinctive markings representing the individual teeth in the associated semi-circular patterns, and a closed population of suspected biters is identified - each with distinctly differing arrangements of teeth - bitemark analysis can be done to reliably identify or exclude given biters in the suspected biter population. These bitemark cases are best exemplified with infant abuse victims where only a small number of known caregivers had contact with and access to the infant when the abusive attack occurred or in sexual assault cases where the sexual assailant was known to the victim. Supporting DNA evidence from swabbings of the bitemark in any given case can further aid in establishing biter identity or exclusion.

Historically, there are two issues in bitemark analysis that have led to the problem bitemark cases; operator bias and analyst error. Operator bias is a well known problem in many scientific investigations. It is incumbent on the operator to be able to know where bias can be introduced in a given case and what steps to take to avoid the introduction of bias, including the use of an independent case review by another qualified analyst.

The problems with incompetency of the analysts have been of far greater concern. Using poor quality bitemark evidence, poor methodology as well as the introduction of the extraneous issues of bias in the investigation, opinions have been rendered in bitemark cases that are not scientifically supported. It has been these cases by a small number of analysts that represent the majority of past wrongful convictions based, in part or completely, on bitemark analysis. Contemporary bitemark analysis includes processes to avoid errors by the analyst before preparing the final bitemark case report.

The 2009 National Academy of Sciences Report, "*Strengthening Forensic Science in the United States: A Path Forward"* correctly identified the problems of bias and analyst error and made further comments on where they felt the process of bitemark analysis should improve. However the report completely neglected to mention where bitemark analysis was an appropriate methodology in criminal investigations, where properly applied bitemark analysis had contributed to successful criminal prosecutions in assault and abuse cases and how to strengthen those aspects of bitemark analysis on "…the path forward."

The evolving future of bitemark analysis will include competency testing for bitemark experts, improved guidelines and standards, research in areas of pattern creation and analysis as well as the relationship between the biter's dentition and the patterns created by those teeth when biting living skin.

Bitemark Analysis, Bias, NAS Report 2009

E45 Review of the Scientific Basis of Bitemark Comparison: Pre-NAS Report

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The goal of this presentation is to review the scientific background of bitemark analysis that existed prior to the 2009 National Academy of Sciences (NAS) Report.

This presentation will impact the forensic science community by providing a historical summary of the peer-reviewed literature for this forensic discipline.

The precedent for bitemark admissibility was mainly set in the 1970's with two landmark cases: The first was *People vs. Marx*. In this case, the methodology used in the comparison process was deemed *not novel*. That methodology consisted of the use of dental X-rays, models, and photography. In the second case, *People vs. Milone*, dental individuality, based on the process of victim ID, was incorrectly extrapolated to bitemarks.

The foundation of bitemark analysis rests on the ability to distinguish between dentitions; that is, there are features that make each persons set of teeth different. There is far more information with which to make a comparison in victim identification than in bitemark analysis, as this post-mortem identification involves examination of possible combinations of 32 decayed, missing and restored teeth, including root morphology, trabecular bone patterns and sinus morphology.

With bitemark analysis, typically only the biting surfaces of the six anterior, or front teeth of each arch impress the tissue, severely limiting the amount of information available to claim one dentition is distinct compared to another.

Furthermore, bitemark analysis is often accomplished by evaluating a wound or injury pattern in human skin and comparison of that pattern to representations of the dentition. Human skin is a notoriously poor recording medium, and prone to highly variable distortion. Given the loss of resolution of the resulting pattern due to these properties, the ability to distinguish between individuals is further compromised.

A survey of the peer-reviewed literature concerning bitemarks since the 1960's reveals interesting patterns. There are very few empirical studies that substantiate any scientific basis of bitemark analysis, or that investigate the core premises of dental individuality and transfer of the dental pattern to skin. Most of the publications consist of case reports, review papers, and technique reports on procedural issues and evidence collection.

With regard to the dental aspect of bitemark analysis; that of individuality of the dentition, there have been very few studies in six decades that have attempted to address this issue. Unfortunately, these studies suffered from inappropriate use of statistics, lack of statistics altogether, or had too small of a sample size from which to make a conclusion. With regard to properties of the skin, there have been even fewer studies in the forensic odontology literature.

With such limited scientific basis, it is valid to question how bitemark comparison has reached the level of acceptance that it has, with the serious consequence of determining the life or liberty of an individual. This question has been brought to the fore by the number of recent exonerations of those incarcerated or convicted on bitemark evidence.

Bitemarks, Bitemark Research, History

E46 Review of the Scientific Basis of Bitemark Comparison: Post-NAS Report

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The goal of this presentation is to explore basic research in bitemark analysis published since the release of the 2009 National Academy of Sciences (NAS) Report.

This presentation will impact the forensic science community by providing a summary of the current status of the scientific basis for this forensic discipline.

The 2009 NAS Report was critical in its assessment of bitemark comparison. Lack of research supporting the basic tenets of this technique was noted. The tenets are that the human dentition is unique and that the unique features transfer to the skin; however, at time of publication of the NAS Report, systematic studies supporting these basic suppositions were conspicuously lacking. As per the NAS Report, "the uniqueness of the human dentition has not been established, the ability of the features of the dentition to transfer to the skin has not been established, and the scope or extent of distortion has not been demonstrated."

Admittance of a technique into the courtroom when there are few systematic studies to support its validity can have negative consequences, as exemplified by a number of exoneration cases. When life and liberty are at stake, there is a responsibility to base testimony on substantiated techniques with a clear understanding of the limitations of the approaches used. When testimony is not grounded in systematic scientific studies, interpretation can become subjective rather than objective.

Given the concerns stressed in the NAS Report, and the serious consequences seen in the courtroom, it would stand to reason that research is warranted to evaluate the basis of bitemark analysis. Current research is exploring these questions and the results are pointing to a cautionary view of bitemark analysis as a means of identification. Those results indicate that there is a large degree of distortion possible in human skin and that relatively small collections of human anterior dentitions contain multiple dentitions with differences below achievable measurement resolution. The inability to distinguish between these dentitions creates questions about the notion of uniqueness within this class of forensic data.

Many of the details that make the dentition as a whole distinct for victim identification are not present in a bitemark, creating further limitations on the ability to individuate in this circumstance. As such, it may be more appropriate to consider the extent to which distortion would include similar suspect dentitions in a bitemark, given the loss of resolution encountered in skin, and whether it is still possible to make distinctions in small, closed populations.

It is inevitable that a degree of distortion will be seen in a bitemark. Human skin is a poor recording medium as it exhibits a visco-elastic, anisotropic response to stress. Skin tension lines dictate the extent to which skin can stretch. These tension lines vary across the body and can be altered depending on body movement. Given this property of skin, it should not be surprising to see a large range of distortion. Results demonstrated that the maxillary and mandibular arches distort independently as impressed in a bitemark, meaning that the distortion in one arch is not reflective of the distortion seen in the other. Furthermore, it has been observed that multiple bites, all made with the same dentition will vary widely in the pattern seen, and that other dentitions, which did not inflict the bite, may be a better candidate than the biter.

Bitemark analysis has a long history in the legal system and it is inarguable that mistakes have been made, resulting in incarceration of innocent people. The new level of scrutiny of forensic evidence will demand fresh examination of the scientific basis and admissibility of bitemark comparison.

Bitemarks, Bitemark Research, NAS Report

E47 Report on a Recent *Frye* Challenge of Bitemark Evidence in New York

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The goal of this presentation is to discuss a recent bitemark case and through that discussion offer broader strategies for challenging the admissibility of bitemark evidence. More specifically, the presentation will discuss in detail a recent *Frye* challenge to the admissibility of bitemark evidence, focusing on the defense and prosecution litigation strategies in the wake of the National Academy of Sciences (NAS) Report, "*Strengthening Forensic Science in the United States: A Path Forward*."

This presentation will impact the forensic science community by demonstrating how the exonerations of numerous innocent defendants convicted in part based on bitemark evidence and the release of the NAS Report highlights that forensic odontology has not been validated, as well as the danger of admitting into evidence unvalidated disciplines where life and liberty are at stake. The ability of forensic dentists to consistently and with any degree of accuracy associate an individual's dentition with a bitemark recorded on human skin has never been scientifically validated. Recent research has further eroded the scientific community's confidence in bite comparison as a forensic discipline.

The NAS Report was critical of nearly all pattern and impression evidence, but was particularly critical of bitemark evidence, concluding that there is "no evidence of an existing scientific basis for identifying an individual to the exclusion of all others" by using bitemark comparisons, because there is "a lack of valid evidence to support many of the assumptions made by forensic dentists during bitemark comparisons."[1] The fundamental assumptions – that human skin is a reliable registration material for bitemarks and that the human dentition is unique – have been challenged in research published since the NAS Report.

The pre-trial litigation in a homicide case in New York City presents a unique learning opportunity for judges, prosecutors and lawyers who may be presented with bitemark comparison evidence in a criminal case. Although the defendant was excluded from a DNA swab taken from the bitemark at issue, the opinion of the initial prosecution witness, in his forensic report and evaluation prior to the DNA analysis, was that the bitemark was "caused by the dentition of [the accused]." The prosecution expert in the pre-trial hearing (a different expert), whose testimony related only to the general admissibility of bitemark evidence, concluded that the NAS Report "cherry picked" the research it relied upon and that well-trained forensic odontologists are capable of accurately and reliably associating a known dentition with a bitemark injury. In contrast, the defense witness testimony relied on the results of scientific studies published in peer reviewed journals. These studies, the first post-NAS Report efforts to determine whether or not bitemark associations can be validated as a scientific discipline, have cast more doubt about its validity.

The Innocence Project is actively litigating several cases involving bitemark evidence and the newly created Strategic Litigation Unit is focused on, among other forensic disciplines, forensic odontology. This presentation will be an opportunity to learn and discuss best practices for challenging such evidence.

Reference:

1. National Research Council of the National Academies, *Streghthening Forensic Science in the United States: A Path Forward*. Washington, DC: National Academies Press, 2009, p. 176.

Forensic Odontology, *Frye/Daubert*, Bitemark Comparison

E48 Does Bitemark Evidence Meet Modern Evidentiary Reliability Standards? A Subject Expert Panel Discussion

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After attending this presentation, attendees will be educated on the latest scientific basis and legal admissability status of bitemark evidence.

This presentation will impact the forensic science community by bringing together the most knowlegable experts in the field to debate a subject that is of significant importance to the American criminal justice system.

The question posed to the bitemark subject experts is, "Does bitemark evidence meet or exceed threshold legal admissibility standards?" This presentation will answer questions related to bitemark reliability from the audience.

The admissibility of bitemark evidence historically has been based on courtroom precedence and a half-century acceptance in the underlying fundamental principles of the technique. Over the past decade wrongful convictions based on bitemark evidence have called into question the fundamental scientific basis of bitemark identification methods.

The standard for admission of scientific expert testimony differs from court to court and from state to state. Generally courts are guided by Rule 702 of the Federal Rules of Evidence and two main cases, *Frye v. United States* and *Daubert v. Merrell Dow Pharmaceuticals, Inc.* The *Frye* test for admissibility requires the scientific principle being proffered as evidence "to have gained general acceptance in the particular field in which it belongs." If the trial court determines that a technique has gained general acceptance in its field, then the technique is deemed reliable enough to be admitted at trial. In 1993, the United States Supreme court in the *Daubert* case held that Rule 702 of the Federal Rules of Evidence changed the *Frye* test. Rule 702 states in part, "*If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise . . . ." Daubert* retained *Frye*'s general acceptance principle for admission, it also stated that scientific evidence must be both relevant and reliable. The *Daubert* court went on to outline factors relevant to the admissibility of scientific expert testimony to aid trial court judges, including: (1) the theory or technique must be able to be, and have been, tested; (2) it must have been "subjected to peer review and publication;" (3) the known or possible error rate of the scientific technique must be taken into consideration; (4) the court should take into account the "relevant scientific community" and a determination of the degree to which the theory or technique in question is accepted in that community; and (5) the focus is on the principles and methodology behind the technique, not necessarily on the conclusions generated.

The guidelines of the American Board of Forensic Odontology (ABFO) permit an expert member to render conclusions expressing near certainty – they could conclude that a bitemark matches a criminal defendant to a "reasonable medical certainty" and "high degree of certainty" and/or "a reasonable medical and dental certainty, did inflict the injury." This language appears to mean, in certain cases, a virtual certainty; no reasonable or practical possibility that someone else did it. Does science exist for what observations and analysis could permit an expert to draw such conclusions? The guidelines added that experts may not convey "unconditional certainty;" however, they may express "reasonable medical certainty," and noted that it was acceptable to state that there is "no doubt in my mind" or "in my opinion, the suspect is the biter" when such statements are prompted in testimony. Many expert conclusions that go beyond the ABFO guildline are still allowed, such as that a person "beyond a reasonable doubt" or "99 percent certainty" the suspect made the bite.

Are the ABFO guidelines faulty? Is the underlying method of this discipline reliable?

Bitemark, Frye, *Daubert*

E49 The Amanda Knox Case: Scientific Investigation and Criminal Trial in Italy

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After attending this presentation, attendees will understand the scientific and judicial issues that have characterized the criminal trial held in Italy against Amanda Knox, accused to be the murderer of Meredith Kercher. This presentation will explain the peculiarities of the Italian trial system, the differences with the U.S. trial system focusing on the admission and evaluation of scientific evidence, as well as the role of the so called "independent expert."

This presentation will impact the forensic science community by highlighting the need to improve forensic science performance using good science. This is particularly relevant, as in the case to be discussed, when the trial judge, as the gatekeeper of the judicial system, is facing controversial conclusions from the defendant and the prosecution side that may condition or affect his decision. Thus, the judge needs to appoint a board of highly qualified and independent experts in order to establish which hypothesis is correct and reliable.

This case refers to the murder of Meredith Kercher, a young British student, who was found dead in her apartment in Perugia on November 2, 2007. According to the prosecutor's investigation, the murder was committed by Amanda Knox, Raffaele Sollecito, and Rudy Guede. Knox and Sollecito were indicted on murder charges on October 28, 2008. Guede is found guilty of murder in his fast track trial and sentenced to 30 years. The evidence offered by the prosecution is of circumstantial value. No witnesses were present at the time of the crime. Among the evidence, the most damaging piecies of evidence found during the scientific investigation that pointed to the guilt of Knox and Sollecito, was a hook of the victim's bra and a knife found at Raffaele's house. The first of these two elements (the hook of the bra) is found during the first crime search made by the police the day after the murder, but inexplicably it gets lost and will be found and collected only 46 days after the crime, during new technical activities at the crime site.

According to the prosecution theory based on the DNA analyses made by the forensic science lab of the national police, the hook shows genetic material that matches Sollecito, hence his presence at the crime scene during the murder.

According to the defense theory, the DNA found on the hook is due to contamination occurring within the technical activities made at the scene of crime during those 46 days. The second element (the knife) shows DNA on the blade (that matches the victim) and on the handle (that matches Amanda). According to the prosecution theory, this is the "murder weapon" and Amanda is the person who stabbed Meredith. This item has clear circumstantial evidence weight. According to the defenses theory, the DNA found on the blade is LCN-DNA, thus too low in quantity to support a reliable conclusion, especially if it has to be linked to Amanda's guilt.

The Court of first degree accepts the experts' assessments made by the prosecution about the collection, preservation, and analyses of scientific evidence. Contrary to normal practice within the Italian courts, which usually appoint an independent expert to settle disputes among the experts, the Court rejectes the request made by the defense.

After eleven months of trial, the Court found Knox and Sollecito guilty on all counts in the stabbing death of Meredith Kercher. Sollecito received a 25-year sentence; Knox received 26 years. The Italian

criminal trial system, unlike the U.S., provides two levels of judgment, with the same possibilities.

November 24, 2010 Knox and Sollecito's murder appeal process began. Due to expert disputes arising in the first trial, two forensic experts from Rome's University are sworn in by the appeal court and retested the two crucial pices of forensic evidence used to convict Knox. They gave a second (neutral) opinion about the knife and the claps from Kercher's bra, which was cut from her body during her murder. The goal of the appeal court is to give the right value to those pieces of evidence, within the trial context, according to the most reliable science.

Forensic specialists told the court that DNA evidence linking Knox to the alleged murder weapon was unsound; that while they agree Knox's DNA was present on the knife handle, tests for Kercher's DNA were unreliable. The sample, however, was so small that forensic scientists were not able to double test it in accordance with international forensic science rules, which Knox's legal team says raises doubts about its validity. The Court appointed experts testified that police forensic scientists involved in the murder case made a series of glaring errors during their investigation.

In a point-by-point reconstruction, the experts say that because of the errors made by police during the original investigation, the evidence against Knox and Sollecito should be considered "inadmissible." On October 3, 2011, an Italian jury overturned the 2009 murder conviction of Amanda Knox and Raffaele Sollecito.

Amanda Knox Trial, Independent Expert, Evidence Submission

E50 Parental Alienation Syndrome as it Relates to Hague Abduction Convention Article 13b: To Be or Not to Be Admissible in the Courts

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After attending this presentation, attendees will understand issues surrounding the controversial scientific evidence issues regarding parental alienation syndrome in light of the numerous children involved at international and local levels in these child custody disputed cases.

This presentation will impact the forensic science community by offering diverse and enlightening insights into this controversial scientific evidence issue of the court's admission or exclusion of Parental Alienation Syndrome (PAS) evidence, and by suggesting recommendations as to how courts and lawyers should handle cases involving PAS evidence in light of the numerous children involved at international and local levels in these child custody disputed cases.

Parental Alienation Syndrome (PAS) is a theory surrounded by much debate amongst scientists and lawyers as to its diagnostic legitimacy. Questions concern the scientific foundations, if any, of PAS and its practical applications in legal settings, particularly as PAS affects children and families across the globe. In most instances where a parent abducts a child from the other parent, the Hague Abduction Convention provides for the Court to exercise its discretion to return the child to his or her "habitual residence." However, in certain rare cases under Article 13b, the judge or administrative authority of the requested country (referred to as a State) is not bound to order the return of a child if the Court finds a grave risk exists and to return the child exposes that child to physical or psychological harm. Many States use Article 13b to request psychological profiles, detailed evaluations of parental fitness, evidence concerning the nature and quality of lifestyles and relationships. Clinical examinations and observations of interactions between the parents and the child are pivotal to understanding the family dynamics and unveiling the truth. How are and should these clinical findings, diagnoses and recommendations be implemented and accepted by the Courts across the globe? Is PAS acceptable science? Is a determination of PAS helpful to the Courts? Is their a comfort level achieved by the finder of fact - the judge or administrative body - in utilizing this information? Although parental alienation syndrome has not been recognized or included in Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-4) in the past, experts and others are discussing whether this concept of parental alienation should be included in DSM-5 which is due to be released early next year. What do these discussions encompass on this controversial topic known as PAS? Should parental alienation be considered as a syndrome?

Why is PAS so controversial? PAS, it is argued, lacks an empirical basis as a psychiatric diagnosis. However, Dr. Gardner, the first expert to acknowledge parental alienation as a syndrome in 1985, used the term "empirical" to mean direct patient observation only. Although he used no scientific experimentation, Dr. Gardner claimed he had ample "empirical" evidence to support PAS as a psychiatric syndrome to which he applied a statistical analysis methodology. Other scientists disagree with Dr. Gardner as to whether PAS should be classified as a syndrome and question whether PAS has been subject to peer review; whether PAS is generally acceptability in the field of psychiatry or psychology; and whether PAS has scientific empirical validity as to falsifiability, error rates, etc. Judges across the globe have struggled as gatekeepers of relevant and reliable scientific evidence to admit or exclude PAS evidence in their courtrooms.

Parental Alienation, Child Abduction, Syndrome Evidence

E51 Parental Alienation Syndrome: A Questionable Concept

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After attending this presentation, attendees will understand the issues surrounding the controversial concept regarding PAS, and how it affects the litigants and the judicial processes involved in determining child custody issues.

This presentation will impact the forensic science community by increasing understanding about the diverse perspectives on the admissibility or exclusion of Parental Alienation Syndrome (PAS) evidence. This should enable practitioners to be more competent, aware, and knowledgeable of the complexities of this concept in the performance of their duties in the courtroom. A more balanced and productive perspective on this issue, including the need for more research in this area will be presented. This presentation will address how this concept has become more of an adversarial weapon than a therapeutic tool to assist the families in resolving difficulties during divorce. The assumptions involved with this concept will be examined and the presenters will provide suggestions about the type of research needed in this area and how it can be accomplished.

Experts are testifying in courts across the globe about Parental Alienation Syndrome (PAS) which has become a popular concept used in custody battles; however, there has been significant debate inside and outside the courtroom over the admission of PAS evidence. Is their empirical data to support expert testimony concerning this so called syndrome? Does the concept meet the scientific admissibility standards of either *Frye* or *Daubert*? If these standards are not met, what is giving impetus to the growing use of PAS in the courtroom?

This presentation will address these questions after tracing the beginnings of the use of this concept, which was first presented in 1987, by Dr. Richard Gardner, a psychiatrist who dealt with divorce and custody issues. He defined the phrase as a concept based in part on the idea of "brainwashing," but also involving conscious elements and other factors such as subconscious and unconscious ones existing within a parent which contribute to that parent's negative intentions to

influence the child to reject the other parent. Dr. Gardner believed that a child can become so obsessed with animosity for the alienated parent that the child's animus becomes independent of the alienating parent's contributions. Minor altercations experienced by the child with the alienated parent become the child's justifications for his or her feelings toward the alienated parent.

Dr. Gardner categorized parental alienation cases as fitting on a continuum which included three categories from mild, moderate, to severe. However, his promotion of the concept has raised questions about the basis for his beliefs and opinions. Questions focus on the scientific basis of the concept. How should lawyers and judges view this evidence? Is this concept helpful to the trier of fact? What are the discussions amongst the experts as to the reliability and validity of the concept of parental alienation? Should lawyers and judges scrutinize expert testimony about parental alienation or should they accept this concept as a mental disorder even though there is currently no recognition as such in DSM-4 (Diagnostic and Statistical Manual of Mental Disorders)? What discussions have taken place about this concept's inclusion in DSM-5 which is due to be released in early to mid 2013?

PAS has universal appeal due to claims about the effects of parental alienation on the children experiencing it. Parental alienation has been cited by legal authorities in the United States and also in international jurisdictions and venues where parents abduct a child from one country to another country. In this world of ever-growing litigation, parental alienation as a concept needs to be explored more fully using proper scientific methods. More scientific research in this area can assist the experts and the courts providing appropriate remedies to address the possible harm allegedly caused to a child affected by parental alienation.

Parental Alienation, Child Custody, *Daubert*

E52 Straight Shooters or Hired Guns? Expert Forensic Evidence in English Courts

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After attending this presentation, attendees will gain insight into how forensic experts in the United Kingdom (UK) are trained, how they practice, and how they interact with the legal system.

This presentation will impact the forensic science community by stimulating discussion of the relative advantages and disadvantages of different approaches to both the concept of the expert and the way that expert evidence is weighed and judged by the court.

In the UK, there is no formal process for "judging" the admissibility of expert testimony equivalent to the "trilogy" in United States Law of *Daubert*, *Joiner,* and *Kumho*, and therefore the concept of the expert and the contents of expert testimony are very differently viewed and regulated in the two jurisdictions. The acceptance of expert testimony in English courts is a complex interaction between practical experience (both of the expert's own field of expertise and of giving testimony), observation of the work of other professionals (including observing more senior practitioners giving testimony and observing the testimony of an expert called by "the other side"), knowledge of current best evidence and research, informal teaching and formal training.

In English law, expert witnesses are defined as "witnesses to the court" that is to say the expert is expected to give unbiased testimony no matter whether they are called to give evidence for the prosecution or defense. Many forensic pathologists and indeed experts in other fields accept instruction from both prosecution and defense teams and are regularly called to give evidence by both prosecution and defense barristers. As a result of this, there is much less of a concept of the defense expert as a "gun for hire", as many forensic experts will be seen giving evidence for one side in a certain case, and for the other in their next case. This is generally considered to be a positive aspect of expert evidence, as it reduces the risk of the pathologist becoming excessively "prosecution-centric" or "defense-centric." The forensic expert witness in England is in a highly privileged position compared to other witnesses (eyewitnesses and professional witnesses) and often have extensive interaction with the legal teams who are relying on their evidence before his or her attendance at court which allows the prosecuting or defending barrister (counsel) to "tailor" their questioning to what the expert is confortable to say under oath. To mitigate against this, experts are permitted to listen to another expert's oral testimony, and even advise counsel during cross examination as to which areas of the evidence to explore more thoroughly and which to leave well alone (all the while attempting to remain an unbiased witness to the court of course)!

Finally, the potential problems that arise in any court of how a jury composed of people with no prior expertise in, or experience of, forensic pathology or science can be assisted to make a reasonable decision regarding conflicting opinions expressed by experts offering testimony and the concept of the "joint expert statement" will be discussed.

Expert Evidence, Prosecution, Defense

E53 Which Expert to Believe? Lessons Learned From a Leading English Tort Case

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After attending this presentation, attendees will better comprehend the evidential issues related to experts and scientific evidence in two common law jurisdictions that may differ in detail but still retain much in common after evolving separately for more than 250 years.

This presentation will impact the forensic science community by conveying knowledge, competence and performance about diverse perspectives on these issues that will enable legal practitioners, whether lawyers or judges, to be more competent, aware and knowledgeable about the complexities inherent in judging the merits of the differing strands of opinion evidence that may be offered to the Court by the parties in adversarial proceedings and will thus assist in the performance of their duties inside and outside of the courtroom. The presentation will help forensic practitioners to best assist the Court as experts by exploring the factors that judges have to take into account in choosing one particular expert's opinion over another's.

It is well recognized that the 1997 case of *Bolitho v. City and Hackney Health Authority*, 4 All ER 771, is an important English tort law case on the standard of care required of medical specialists. The case involved the care given to a child with croup and the Court heard differing opinions about whether and when the patient should have been treated by endotracheal intubation. The House of Lords held in *Bolitho* that there must be a "logical basis" for a medical expert's opinion. The Court arrives at such a conclusion by weighing risks against benefits, and judges have the authority and responsibility of choosing between two bodies of experts and of rejecting the one the Judge considers "logically indefensible." Leading authorities in this area of English tort law have interpreted *Bolitho* as establishing the precedent that the Courts set the law, not the experts. This presentation will discuss both the impact and aftermath of *Bolitho* from both English and United States perspectives. This presentation will also give consideration to *Bolitho* in terms of factors derived from two very important leading cases on evaluating scientific evidence in federal and state courts in the United States, *Daubert and Frye*. Federal Rule of Evidence 702 on expert testimony will also be discussed as to the reliability of the "scientific knowledge" grounded in scientific methodology and connoting a body of known facts accepted as true on good grounds and as to the relevancy of this evidence as having a valid scientific connection to the pertinent inquiry in court. The Federal Rules of Evidence on Expert Evidence will be compared and contrasted with the English Civil Procedure Rules (Part 35 – Experts and Assessors) and the Law Commission Report No. 325 "Expert Evidence in Criminal Proceedings in England and Wales." The Law Commission is a statutory independent body created by the Law

Commissions Act 1965 to keep the law under review and to recommend reform where it is needed. The aim of the Commission is to ensure that the law is fair, modern, simple, and as as cost effective as possible.

Experts, English Tort Cases, *Daubert-Frye*

E54 Why and How — Reforming Medical Liability in Psychiatry

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After attending this presentation, attendees will understand some of the principles of the medical liability in psychiatry, a field in which reaching the evidence of legal responsibility is particularly hard.

This presentation will impact the forensic science community by presenting a possible solution for reforming medical liability in psychiatry.

The necessary respect of the medical activity of the psychiatrists implies that their liability, as for other doctors, could be declared only in presence of convincing evidence both of negligence and of causality; however, reaching such evidence is extremely difficult, for many specific reasons. First, not every failure in judgment can be automatically translated into negligence, as there is negligence only when there is "a failure to meet the standard of care."

Furthermore, the predictability of the auto- or hetero- offensive acts must be evaluated *ex ante,* in concrete terms and cannot be deducted, in general and in theory, from the diagnosis. It would be improper to consider suicide as predictable in all patients affected by major depression or to assume homicide as predictable in all chronic schizophrenic patients. It is especially necessary to avoid being influenced by facts which occurred after the psychiatrist treatment.

Therefore, in order to avoid subjecting the psychiatrist to an excess of review, it is essential to interpret the predictability in a strict manner, by considering only those circumstances as foreseeable in which the clinical history and the specificity of the moment make to reasonably assume the harmful event as probable and not only possible in theory.

Moreover, the predictability requirement cannot be automatically inferred from the state of unsound mind of the murderer. Such condition could derive, for example, from an unforeseeable and sudden flaring up of the pathological state, linked to some peculiar events in the patient's life of which the psychiatrist was unaware (e.g., a death in the family, the loss of job by a person that was under therapy but able to normally work). Such an event could provoke an unforeseeable and uncontrollable reaction.

Furthermore, when there is not a total state of unsound mind, the homicidal or suicidal behavior can't be evaluated as exclusive expression of the psychopathologic state and therefore no certain judgment could be formulated concerning the foreseeability of the event from the doctor, just because the patient's conduct was not entirely "pathologic," but was also an expression of his/her self determination ability, even if partial.

Finally, the need to protect the freedom and the dignity of the patient prevents the ability to control him/her physically and pharmacologically, with the exception of rare cases. Therefore, it is often impossible to avoid the harm.

Because of the difficulty in demonstrating liability in psychiatry, in some cases the Supreme Italian Court tends to reduce the degree of proof necessary to convict the doctor. This solution is questionable because it compromises the professionalism of the psychiatrists and reduces their constitutional right of defense. On the other side, applying rigorously the rules of medical liability also in psychiatry would reduce the number of convictions and this could induce psychiatrists to have less careful conducts towards the patient's interest.

In order to prevent this risk, it appears necessary to create a statute that, on the one hand, binds the health facilities to provide doctors with more strict guidelines and, on the other hand, increases the power of the health facilities to sanction imprudent doctors independently from the occurrence of damage and the presence of causality.

In this way, the protection of the patient's health is reinforced through the prevention of negligence rather than through the compensation of damages. As a consequence, a general reduction of the legal proceedings is also obtained, improving the quality of clinical practice.

Psychiatry, Medical Liability, Reform

E55 Exposing a Document Examiner's Flawed Analysis in a $300 Million International Bank Fraud Case

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After attending this presentation, attendees will: (1) have a greater appreciation of the need to use a competent board certified Forensic Document Examiner (FDE) at the outset of any case dealing with a questioned or disputed document and will know more about how to find and select such an expert; (2) learn about some of the basic examination procedures used by FDEs to detect altered documents and how such evidence is effectively presented in court; and, (3) learn the distinction between various certifications claimed by forensic experts, how to evaluate the stated credentials of a given expert, and what information contained on an expert's resume should be clarified before hiring an expert.

This presentation will impact the forensic science community by providing all stakeholders in the administration of criminal or civil justice – experts, lawyers, and judges – with a better understanding of the need for more meaningful training, certification, and proficiency testing of forensic experts.

The case presented highlights the need to have meaningful training, certification, and proficiency testing of forensic experts and why lawyers must exercise greater due diligence when selecting an expert.

In 2006, a lawsuit was filed in U.S. District Court, SDNY against the largest bank in the Philippines, to enforce a "Manager's Check" in the face amount of twelve billion pesos (equivalent to 225 million U.S. Dollars) that was allegedly issued by one of its municipal branches on March 21, 2000; the lawsuit also sought damages of $75 million dollars, representing interest accrued since the negotiable instrument was issued. In two well-written decisions, U.S. District Court Judge Sheira Schindlin initially granted the bank's motion to dismiss the complaint on the ground of *forum non conveniens*; several months later, upon a motion to reargue, reversed the earlier dismissal and determined that the trial of the action should be heard in the United States and not the Philippines.

The Plaintiff retained a document examiner with twenty years of experience in federal and state crime laboratories to examine and determine the authenticity of the Manager's Check in dispute. The Plaintiff's document examiner was not a Diplomate of either the Board of Forensic Document Examiners (D-BFDE) or the American Board of Forensic Document Examiners (D-ABFDE), both FSAB-accredited boards; nor was he a member of the American Academy of Forensic Sciences (AAFS). In addition to claiming expertise in document examination, the Plaintiff's expert claimed expertise and certification in fingerprint identification.

After examining the original Manager's Check and an original specimen of a contemporaneously issued "Cashier's Check," the Plaintiff's document examiner submitted a written report describing the results of his microscopic and optical examinations of the original documents submitted to him, opining that the "Manager's Check" was "consistent with being a genuine instrument" and "displayed no irregularities." Thereafter, a board certified forensic document examiner (D-BFDE) with more than thirty years of experience was hired by the defendant bank to examine the very same documents.

In this presentation, the fascinating background and intrigue surrounding this federal court case will be relayed and the actual

photographic and digital evidence presented to U.S. District Court will be displayed. It will be illustrated how the forensic examinations of the very same documents examined by the Plaintiff's expert revealed significant irregularities that led to irrefutable, demonstrative proof that the Manager's Check at issue was an outright forgery. The proof presented, which was subsequently confirmed by another board certified document examiner (D-ABFDE) retained by Plaintiff's counsel to examine the evidence and review Mr. Sulner's findings and conclusion(s), will illustrate how a standard bank check was altered and converted into a purported official bank check and negotiable instrument. Using this case as an example of how poor forensics can adversely affect the administration of justice, the need for more meaningful training, certification, and proficiency testing of forensic experts, why lawyers need to exercise greater due diligence when selecting an expert, and how lawyers should go about selecting an expert will be discussed.

Flawed Document Exam, Exposing FDE Error, Flawed FDE Findings

E56 Results of the FBI Laboratory's Evaluation of Compositional Bullet Lead Analysis Testimonies

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After attending this presentation, attendees will understand the process undertaken by the Federal Bureau of Investigation Laboratory in evaluating examiner testimony when results from Compositional Bullet Lead Analyses (CBLA) were used in court. The results of the review will also be presented.

This presentation will impact the forensic community by presenting a model to consider when the testimony of expert witnesses in other fields may require evaluation.

CBLA was a forensic discipline used by the FBI Laboratory for over four decades when either a firearm was not recovered or a fired bullet was too mutilated for microscopic comparison of physical markings. If crime scene bullets were "analytically indistinguishable" to other bullets, research suggested that they likely originated from the same source of molten lead. Thousands to millions of bullets may be produced of the same composition and are usually packaged within the same box and in other boxes of the same caliber and type over a relatively short time period.

In 2002, the FBI commissioned the National Academy of Sciences (NAS) to evaluate the scientific basis of CBLA. Specifically, the NAS was asked to assess and provide recommendations for future improvements in three areas: (1) the analytical method in use at the time; (2) the way that the FBI was conducting statistical comparisons of the analytical results; and, (3) the appropriate statements that can be made in interpreting the results of a CBLA comparison.

The NAS released their report, *"Forensic Analysis: Weighing Bullet Lead Evidence,"* in February of 2004. The report contained 22 findings and recommendations. Upon issuance of the report, the FBI Laboratory temporarily suspended all CBLA examinations in order to review and evaluate the report's recommendations. Over the next 15 months, all recommendations were implemented and a revised analytical protocol was developed and revalidated. But in mid-2005, the FBI Laboratory decided to permanently discontinue the CBLA examination due to conflicts in the recommended statistical techniques suggested in the report, lack of knowledge of geographical distribution data, and the media labeling the examination as "junk science." The FBI Laboratory affirmed that previously issued reports were not in error and the foundation of CBLA was valid. Nonetheless, notification was made to the FBI's case contributors, the National District Attorney Association, the National Association of Criminal Defense Lawyers, and the Innocence Project of the decision to discontinue the CBLA examination.

In late 2007, the FBI agreed to undertake an extensive review of CBLA testimony offered by its examiners in all criminal cases that could be identified. Over the next four years, over 2,200 case files were reviewed to allow for requests to be made for testimony transcripts.

Each CBLA testimony was evaluated following a process developed by the FBI and the U.S. Department of Justice with input from the Innocence Project. Testimonies were deemed "inappropriate" if they fell into one of three categories: (1) if at any point during the testimony, the examiner suggested that a crime scene bullet could be linked to single "box" of bullets to the exclusion of all others; (2) if at any point during the testimony the examiner made some other statement that overstated the significance of bullets being "analytically indistinguishable"; or, (3) if the examiner failed to provide information that there would be a large number of other bullets, unrelated to the case, that would also be "analytically indistinguishable" due to the bullet manufacturing process. Testimonies that were not deemed "inappropriate" using these criteria were, by default, classified as "appropriate."

CBLA testimony from 232 transcripts were evaluated. Using the above criteria, 150 of the testimonies were deemed inappropriate, while just 82 were appropriate. While these results were surprising, the stringent review process ensured that the evidence was not misconstrued by the courts or juries. The ramifications and lessons learned from the CBLA testimony reviews will be discussed.

Bullet Lead, Testimony, Review

E57 The Challenges Facing International Victims of Sexual Gender-Based Violence

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The goal of this presentation is to educate the forensic DNA community on the judicial challenges faced internationally with the acceptance of DNA evidence in court.

This presentation will impact the forensic science community by detailing the powerful story of the adjudication of an international sexual assault case and advocating for judicial evolution across the world with the increase in the use of forensic evidence for such cases.

As a U.S. citizen who worked and lived in Morocco from 2008 to 2009, co-author Ms. Erin Helfert was raped by a Moroccan citizen in 2009, after which time she left Morocco. The clothing from the crime was sent to a laboratory in the U.S. for initial analysis, where semen was identified and samples of the clothing were then subsequently sent to the DNA laboratory in Morocco for testing. The equivalent of a Moroccan District Attorney asked the suspect to submit a buccal swab, which was provided. The Moroccan DNA Laboratory agreed to perform a comparative DNA test between the semen stain from the victim's clothing and the buccal sample from the suspect. Subsequently, a DNA match was found between the semen on the clothing and the suspect's DNA. The judge presiding over the case agreed to consider this as evidence, which represents the first time DNA results would be used in a sexual assault case in Morocco.

The plight faced in this case underscores a problem that may be faced by international travelers when visiting and/or working in foreign nations in which the application of forensic science is either in its infancy or not routinely applied. Due to great persistence and self advocacy, this case has established a series of legal precedents in Morocco and can be used as an example for other nations. Among these, this criminal rape case is the first in which forensic evidence has been considered by both the Moroccan equivalent of the district attorney and the judge. In light of forensic evidence, the case evolved from a hearsay situation to a legitimate criminal charge in the eyes of the Moroccan authorities.

Many times travelers and expatriates encounter judicial systems and standards of practice that are markedly different that those in their home nations. For victims of crime, these travelers often face cultural differences that impact the justice and resolution of each case as well as the ability of the judicial system to benefit from forensic analysis. This particular case represents not only an example of an individual's pursuit of justice, but also demonstrates how such cases can spur national judicial evolution and the increased application of forensic science in the

courtroom. It is also an example of how an individual can make a difference on a national scale and the lessons learned by pursuing justice in this case can serve as models for other nations.

The victim will be present in Morocco during the trial and will be involved in the courtroom proceedings. The introduction of DNA test results in Morocco and the ability of both the defense and prosecution to effectively argue their sides of the case in front of the judge will be precedent setting. A guilty conviction, supported by DNA evidence, will be a gain in a country where the conviction rate of rape cases is currently only six percent. The results of this case should have a significant impact in Morocco on the prosecution of future rape cases and thus may have a major cultural impact on a nation and serve as an impetus for greater reliance on DNA testing for rape cases.

Gender Violence, Sexual Assault, International Crime

E58 Court-Assessed Fees May Create Bias in Forensic Science

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After attending this presentation, attendees will recognize that funding sources and methods constitute a potential source of bias in forensic science. Attendees will understand some of the mechanisms linking court-assessed fees to bias in forensic science analyses and interpretations. Attendees will learn that court-assessed fees are charged to defendants upon conviction and that persons found not guilty are not subject to them. Attendees will learn how several states have statutes mandating the partial funding of public crime laboratories through court-assessed fees. They will learn which 15 states have such statutes. Attendees will learn that in other jurisdictions court-assessed fees may help fund public crime laboratories as a matter of policy rather than state law. Finally, attendees will learn that a fee-based system in which fees are not contingent upon the outcome of the trial or of the forensic examination would reduce or eliminate the specific potential for bias created by court-assessed fees.

This presentation will impact the forensic science community, including forensic scientists, crime laboratory managers, prosecutors, and public defenders; because they all share a common interest in ensuring that forensic science analyses and interpretations are correct, objective, and unbiased.

This presentation's hypothesis or proposition may be expressed in one sentence. Court-assessed fees may create bias in forensic science analyses and interpretations, whereas non-contingent fees would present a much smaller risk of inducing such bias.

A brief synopsis of the content of the presentation emphasizes the incentive to convict rather than to distinguish between guilt and innocence. When crime laboratories are funded in part by court-assessed fees, lab funds are positively correlated to conviction rates. This correlation creates an incentive to convict rather than to correctly distinguish between the guilty and the innocent. This incentive to convict may induce unconscious bias in forensic-science analyses and interpretations. Non-contingent fees do not create a specific incentive to convict and are therefore less likely to induce unconscious bias.

The presentation suggests, as a general statement of conclusion, the importance of examining the methods of funding crime laboratories from the perspective of their potential to induce unconscious bias in forensic-science analyses and interpretations.

This presentation will impact the forensic science community by identifying a previously unrecognized source of potential bias in forensic science analyses and interpretation. The 2009 NAS Report, "*Strengthening Forensic Science in the United States: A Path Forward*" emphasized the importance of minimizing the risks of bias in forensic science. The report identified several potential sources of bias, including the organization of crime laboratories under law enforcement agencies. The report gave no direct attention to the potential of crime-laboratory funding methods to induce bias and it did not consider the role of court-assessed fees in the criminal justice system. If funding methods are a potential source of bias in forensic science analyses and interpretations, public officials, crime-laboratory directors, and other members of the forensic-science community should seek alternatives to the use of court-assessed fees to help fund publicly-funded crime laboratories. The possibility of moving to non-contingent fees to help support public crime laboratories should be reviewed by the forensic-science community.

Court-Assessed Fees, Bias, Funding

E59 How Italy Deals With the Increase of Medical Malpractice Claims: The Binding Mediation and the Gap in Understanding Between Patient and Physician

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After attending this presentation, attendees will understand some of the legal aspects of a mandatory mediation system recently introduced in Italy regarding medical malpractice litigation, how medical liability claims are handled by the mediator, statistic data collected by the Italian Ministry of Justice, potential benefits for physician-patient relationship, and differences between two examples of claims settled by mediator and court.

This presentation will impact the forensic science community by providing a model to drastically reduce the number of disputes in court and to promote the dialogue between physicians and patients. In fact, the traditional legal system emphasizes the physician-patient relationship and raises behaviors as reticence and retraction, delaying the achievement of the injury compensation and increasing the defensive medicine.

A new Italian law enacted March 21, 2010 enforces parties, in a medical malpractice action, to proceed with mediation before moving forward with the lawsuit. In the event the parties do not proceed with mediation, the court has the duty to suspend trial, ordering them to try to settle the claim through mediation. A fine is ordered to be paid by the party which does not take part in mediation. The law provides that the mediation must be completed within four months after the patient filed the claim, regardless of the achievement of agreement. If parties do not reach an agreement, the mediator can make a proposal on how he/she would settle the claim and, thus the parties can move to court to resolve their disputes. During the trial, the judge could discretionally evaluate the mediator's proposal. The high cost of mediation is split between plaintiffs and defendants and it is calculated regardless the requested amount for injury compensation claimed by patient. The mediation allows patient to hear explanations behind medical errors or complications and to hear a physician express apology. In the current practice, the mediator does not strictly ascertain the physician's fault by the evaluation of standard of care, but rather he/she promotes and facilitates the communication between patient (plaintiff) and physician (defendants) in order to achieve an agreement that meets their wishes. Parties must be assisted by a lawyer. The mediation is not overseen by court nor does the mediator have to be a judge. The mediator could evaluate the opportunity to be supported by a forensic scientist or specialist, charging fees to parties.

Two case studies will be highlighted, the first one settled by mediator and the other one by the court, comparing the dynamics, length, effects (on the patients' and physicians' quality of life), and the modalities of involvement of forensic scientist.

The impact of the new reform in the field of medical malpractice is also evaluated and discussed on the basis of statistic data collected by the Italian Ministry of Justice. The statistic survey was completed on March 31, 2012. The data confirm relevant decrease of medical liability

claims in front of courts. Moreover, a widespread skeptical attitude towards mediation was registered. Three main criticisms could be identified: (1) the compulsoriness of mediation; (2) the fact that mediation implies expensive costs for parties (remuneration of mediator, lawyer and forensic scientist) without guaranteeing a good compromise; and, (3) the concern in delaying the achievement of injury compensation in case of lack of agreement.

Overall, taking into account that the priority in medical malpractice litigation should be reduce the gap in understanding between physicians and patients, the Italian experience provides a practical example, which deserves international attention in order to discuss and argument whether communication approach could be incentivized by a binding system.

Medical Malpractice Litigation, Mandatory Mediation, Physician-Patient Relationship

E60 Issues in Age Estimation in Medicolegal Cases: Our Experiences in a Tertiary Care Hospital in India

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After attending this presentation, attendees will understand some of the principles on methods of age estimation, as well as daily dilemmas faced by forensic doctors in India, owing to a huge burden of medico-legal work supplemented with poor or lack of documented evidence for age of an individual. Authenticated birth certificates, school documents when available, are admissible in the courts as proof of age, and are considered to be superior to the age estimated through medical opinion. The courts rely on the medical opinion whenever there is disagreement on the documented evidence for age or in those cases, where they are completely missing. As the age progresses, the estimation of age-range also widens considerably.

The presentation will impact the forensic science community by sensitizing attendees toward the medicolegal issues that arise in determination of a person's age, hoping to convey that the technique is always a multi-disciplinary approach involving clinical, dental and radiological examination, but with individual limitations for each of these criteria.

Identification of the living person and the dead is of paramount importance for variety of reasons in forensic practice. While age estimation of unidentified corpses and skeletons for identification purposes has a long tradition in forensic sciences, age estimation of living persons have formed a relatively recent area of clinical forensic medicine which are becoming increasingly important. Not only it is important in the identification of victims and accused in criminal cases, but also in civil cases like marriage, inheritance of property, insurance claims and missing persons etc where determination of age is questionable.

Durnig day to day practice, it becomes challenging for a forensic doctor to pin-point and accurately comment upon the age of an individual, especially when the court of law needs specific age of either parties for fixing the level of punishment and dispensing the case. This presentation details cases referred from the courts, for age estimation of those in conflict with the law, who were pleading innocence on the grounds of age, stating to be juveniles at the time of committing the offense. Many of the crimes were committed by the accused years earlier and the trial took another set of years to complete, further complicating the issue of age. It was noteworthy, that most of the accused were related to cases of heinous crimes like homicide and wanted to benefit from the Juvenile Justice (Care and Protection of children) Act of India; 2000. The honorable court then asked for medical opinion, regarding the bone age of the individual through a board of doctors in the tertiary care hospital.

In practice age estimation. it is always a multidisciplinary approach involving clinical, dental, and radiological examination; however, there are individual limitations for each of these criterions. A careful scientific co-relation of various findings present during examination, when interpreted with the available background history will ultimately solve the problem. This preseneation shall share experiences of formulating medical opinion on estimation of age using various parameters, citing discrepancies in the available review of literature and the legal point of view of Indian courts, regarding age estimation popularly known as "ossification test" among jurists in India.

Age Estimation, Medicolegal, Courts India

F1 Ultimate Dental Chart for Medical Examiners

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After attending this presentation, attendees will learn of a new dental chart that will assist dentists, medical examiners, and law enforcement officers in the proper collection of dental evidence.

This presentation will impact the forensic science community by informing forensic dentists who participate in the charting of the dentition of unknown remains in finding a complete collection of data on one chart in an organized form. This chart should enhance the ability of the dentist or medical examiner to make more accurate conclusions regarding the identification of victims or suspects whose identity has not been established.

Not all medical examiner departments have the services of an ABFO forensic odontologist available on staff. Many are fortunate to have a dentist on call that may assist in the recording of dental evidence in the case of unknown remains. Some medical examiners have to take it upon themselves or enlist the aid of a forensic anthropologist to record the dental evidence. In any case, the medical examiner's department is in need of standardization of dental charting, in the collection of dental evidence, at the time of the dental autopsy. This chart should be clear, as well as comprehensive, and lead the investigator in the correct direction. Another important element is the ability of every satellite medical examiner's department and investigative agency to read and understand what has been recorded by their colleagues. Every medical examiner's department, as well as every dentist, has his/her own charting system, which only contributes to the confusion and the inaccuracy of dental charting. Standardization of dental charting should lead to easier computerization of dental charting in the distribution of evidence among missing and unidentified persons. Standardization also leads to better communication when we are all reading from the same page. This presentation of a dental chart contains the ultimate in knowledge required for a complete dental examination. The Universal Tooth Numbering system, as well as the Federation Dentaire International (FDI) system, are both employed in the permanent as well as the deciduous dentitions. Both systems are the most common means of reference nationally and internationally, and both systems are found in close proximity to the teeth being recorded in this chart. The Universal Tooth Numbering system is in use practically throughout the United States and is the standard system taught in dental schools in this country. The FDI Tooth Numbering System is most popular throughout the rest of the world and is an important element contained within this chart. Although Canada and INTERPOL rely on similar systems, it would be advisable to coordinate these systems into one compatible system that would not rely upon language specificity but would have a more universal appeal and character.

The coding for the identification and condition of individual teeth is charted by the WIN ID system. Although this may be the most popular system, it is not universally employed throughout the world. An odontographic representation of all teeth is available on the front of the chart to note the outline of restorations and/or special conditions. This is the same system used by National Disaster Medical System teams and is easily converted into NCIC codes, which is the system used by the U.S. Department of Justice and the Federal Bureau of Investigation. All WIN ID and NCIC codes are defined within the body of the dental chart. There is a guide to the standard series of dental radiographs and a recommended outline for dental photographs available on the back of the chart.

In addition, there are areas on the chart to note the Classification of Occlusion and a Classification of Remains. Separate areas allow for remarks, dentures, and pathology. The front of the chart contains space for the possible name, age, sex, race, medical examiner, number, and date, together with an area for the signature and license number of the examining odontololgist.

Ultimate, Dental, Chart

F2 3D Photography of Bitemarks and Digital Comparison With an Individual's Model

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After attending this presentation, attendees will learn how an existing technology could be applied to forensic odontology and specifically, bitemark analysis.

This presentation will impact the forensic science community by demonstrating an established modality that can be applied to forensic odontology for more accurate photo documentation of human bitemark injuries. Accurate evidence collection is the first step to improved scientific analysis.

Photographs of bitemark injuries in 2D are the standard methodology utilized to document the injury for future analysis. Due to the curved anatomical surfaces on which many bitemarks occur, such as the human arm and breast, photographic distortion can be introduced as a factor complicating the analysis. This distortion, once introduced, is difficult to rectify without potentially creating further image distortions. Utilizing the advanced technology of 3D photogrammetry systems, this photographic distortion can be eliminated, leading to higher accuracy at the data collection stage.

3dMd is a high-precision 3D surface imaging system with current applications for both living and non-living subjects. The use of the 3dMd system and its precision and accuracy has been established in the scientific literature in sources such as: the American Journal of Orthodontics & Dentofacial Orthopedics, the International Journal of Oral and Maxillofacial Surgery, Facial Plastic Surgery Clinics of North America, and the Journal of Prosthodontics. The imaging system is currently in use in various fields ranging from anthropology and biometrics to craniofacial and cleft lip and palate treatments. Applying this technology to the capture of the bitemark injury could both improve the documentation of the injury as well as allow for 3D bitemark analyses to be performed.

Utilizing a digital imaging system, a digital model of an individual's dentition can be produced in Stereo Lithography (STL) format. STL is a file format native to the stereo lithography CAD software created by 3D systems. STL files describe the surface geometry of a 3D object such as an individual's dentition without any representation of color or texture. Current applications in the field of orthodontics allows STL files generated from cone beam computed tomography of a patient's craniofacial system to be "mapped" to the 3D photograph of the subject's face, allowing for comparison of hard and soft tissues without distortion or magnification.

Applying this same concept, the STL of an individual's dentition could be "mapped" to the 3D surface characteristics captured in the 3dMd photograph of the bitemark. In this manner, a 3D bitemark analysis protocol could be developed. This new protocol would improve upon the current industry standard 2D analysis by minimizing or eliminating photographic distortion.

The digital model technology and STL file capabilities exist today in an applied format; however. the 3D photogrammetry technology must advance to a more portable format in order to have a practical forensic application. This does not preclude the importance of studying the

technology's application to forensic odontology and beginning a scientific endeavor to apply this technology.

Forensic Odontology, Bitemark Analysis, 3D Photogrammetry

F3 Retained Bullet Fragments in the Maxillofacial Region

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After attending this presentation, the attendees will recognize the radiographic appearance of bullet fragments in the maxillofacial region, obtain relevant history in a living subject, recognize the current and possible future morbidity associated with the condition, and be cognizant of the management challenges and options in the living subject.

Conflict and the use of ballistic projectiles including guns is an all-too-frequent occurrence in the society in which we live. Crime solving and management of injuries and the resultant complications is a complicated process. The proximity of vital, special sensory structures, the brain, and the gnathic apparatus makes this process a veritable challenge when the incident involves the head and neck. This presentation will impact the forensic science community by bringing about an awareness of potential injuries to the facial skeleton, soft tissues, and teeth. In addition, the presentation will also describe implications of ferromagnetic effect of retained bullet fragments on specialized imaging procedures.

It is proposed that forensic odontologists, oral and maxillofacial radiologists, head and neck surgeons, and dental surgeons recognize the implications of retained bullet fragments in the facial region and plan investigations, intervention, and reconstruction, while bearing in mind the potential complications.

Synopsis of Content: Three cases of gunshot injuries demonstrating the radiographic presence of retained bullet fragments as noted on panoramic X-rays are presented.

Case 1: A 30-year-old male presented to the dental department with a need to replace his maxillary obturator appliance because it was ill fitting and frequently dislodging from the palate. He had borne a gunshot to his face in 1996. He had undergone several surgical corrections of resulting facial deformities. The injury had laid him legally blind and with episodes of memory loss. The panoramic radiograph showed numerous, discrete, opaque, metal fragments of varying size and shape on the right side overlying the anterior ramus, condyle, disintegrated right maxilla, as well as in the left pterygoid space, and superimposing over the left orbit.

Case 2: A 46-year-old male presented to the dental department for consultation about his teeth. Intraoral examination revealed that he occluded only on two teeth. His history was significant for a gunshot wound that he had endured approximately 20 – 25 years ago. He had then declined reconstruction of his jaw and teeth. Apparently, at that time, he was too overwhelmed with his situation as well as the number of surgeries that he was subjected to, to care about his dentition. Regretting that decision, he was now interested in getting his teeth "repaired." A panoramic radiograph showed numerous bullet fragments within the soft tissue of the right face and pterygomandibular and infratemporal space. The right mandible was fragmented, partially resorbed and the replacing soft tissue was peppered with opaque bullet fragments. Some pieces were also seen within the right upper neck.

Case 3: A 50-year-old male presented for oral rehabilitation and replacement of missing teeth. His history was significant for three gunshot injuries to the face in 2008. The patient's right eye was lost. A panoramic radiograph revealed efforts at reconstructing the right and left maxilla, left palate, and floor of the orbits. His left coronoid process of the mandible was lost and several opaque bullet fragments were noted within the left maxillary antrum as well as in the left infratemporal space.

The implications following the incident, including trauma, disfigurement, fragmentation of the dento-maxillofacial apparatus, soft tissue injury, potential for infections, proximity to or involvement of major blood vessels with the potential for hemorrhage as well as the ferromagnetic interference while imaging, implications of nerve injury, elevated blood levels of lead, and the moving bullet syndrome, are discussed.

General Statement of Conclusion: Gunshot injuries and resultant retained bullet fragments present with life-threatening complications. Several special sensory organ structures, large vascular channels, neuronal tissue, teeth, the spine, as well as soft tissue lie in the potential path of the bullet fragments. Investigation and management of head and neck gunshot wounds are very complicated and challenging procedures. An interdisciplinary approach in a specialized trauma center is warranted.

Gunshot, Fragment, Maxillo-Facial

F4 The Identification in 2011 of 77 Victims After the Bomb Attack on the Government Buildings in Oslo, Norway, and the Shooting at a Youth Camp at Utøya, Norway

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After attending this presentation, attendees will understand the importance of good Antemortem (AM) and Postmortem (PM) information for correct identification of all victims in two tragedies.

This presentation will impact the forensic science community by discussing how a broad and thorough examination of all bodies should be completed. A small detail may be the essential element to establish identity. Identification cannot be made without comparative AM material and dental information may be just as essential as DNA and fingerprints and, together with medical and police evidence, help to secure a correct identification.

On July 22, 2011, after several years of preparation, 32-year-old Anders Behring Breivik, placed a van with 950kg of explosives outside the government buildings in Oslo. The buildings included the offices of the Norwegian Prime Minister and Minister of Justice. Several buildings were almost com destroyed, but only eight people were killed. Approximately two hours later, Breivik, in homemade police uniform with guns and ammunition, ordered a boat to take him to Utøya, an island on the lake Tyrifjord approximately 40km to the west of Oslo. On the island, he shot 69 young people from the Labour Party's youth camp. He later argued that the reasons for his actions were to save Norway and Europe from the Muslims. The relatives and public expected an almost immediate identification of the victims. This required good AM and PM information for comparison. The Norwegian Identification Commission connected to National Criminal Investigations Service (Kripos) in Oslo, is responsible for identifications in such disasters. Because this was a criminal offense, a full autopsy was carried out on all victims. The victims from the government buildings and the streets nearby were severely mutilated by the explosion. Many of the victims from Utøya were shot in the head and face. During the autopsies, two dental teams consisting of either two or three dentists served five autopsy teams. All victims were subjected to a systematic examination including full registration of dental status. Digital photographs and radiographs were taken by the same dentists who did the examination using a Nomad handheld X-ray machine. Documentation of injuries from the shooting was important for the reconstruction of the event. Manual registrations of PM records were made on Interpol forms during the autopsy at the Institute of Forensic Medicine and later transferred to the PM forms in the computer program DVI System International (Plass Data) at the ID-center at Kripos. Photographs and radiographs from each victim were also included in the program.

AM information was transferred directly to the AM forms in the computer program for comparison. Computers aided the search for

identity and facilitate printing professional comparison reports to be included as documentation of the identification.

In this incident, collection of comparative DNA samples wase made at the Relative Centers and this enabled a rapid identification by DNA. Dental records from 57 victims (74%) were collected. Once identity was established further search for dental records was stopped. For 31 persons (41% of the victims) identity was established by dental means. This constituted 54% of those with available dental records. This was higher than expected as the average age of the victims at the time of the shooting was 18 years and many of them had no or few fillings. The identification of all 77 victims was concluded in six days using all methods and combinations of methods for identification.

Terror Attack, Identification, ID-Commission

F5 Missing People and Nameless Cadavers: Implications for Human Rights and Forensic Odontology

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After attending this presentation, attendees will have an understanding of the issues arising from common psychological conditions of family members of missing people.

This presentation will impact the forensic science community by suggesting the importance of maximizing the efficacy of postmortem collection and recording by following Interpol directions and putting greater emphasis on the role of the forensic odontologist.

The non-profit association, "Penelope," created in 2002 with the sole purpose of pursuing social solidarity, people, and dignity, focuses particular attention on the issue of missing people. With expert help, it supports families by organizing conferences and publishing information, aiming to raise public awareness, so that missing people are not forgotten.

Among the goals of the organization is that of the promotion of suitable instrumentation for the collection and elaboration of data regarding missing people in both Italy and abroad, acting as a connection between other organizations and both national and local governmental authorities.

In 2011, the "Penelope" Association created a *pro bono* legal assistance service, made up of lawyers and forensic experts, supporting families looking for their missing family members. In order to offer psychological assistance to families living the painful experience of the disappearance of a loved one, cooperation with the association "Psicologi per i Popoli" (*Psychologists for the People*) was also set up.

The Italian data is disconcerting: approximately 25,000 people have disappeared since 1974, with an annual increase of 800 – 1,000 people. Out of these, 1,651 are Italian children and as many as 8,153 are foreign children. To date, 832 bodies have been found and still await identification.

In 2006, the regional branch of "Penelope" was founded in Apulia. Since 1974, a total of 1,702 people have been reported missing and a total of 53 cadavers currently await identification.

In order to describe common psychological conditions, some cases of missing people in Apulia have been included: for family members of missing people, time becomes suspended and indefinite, and there is a gradual confusion regarding the perception of time as well as disruption of the sleep-wake rhythm. Everyday activities, routines, and work engagements are all suspended, as the return of the missing person becomes top priority. The absence of a person due to uncertain causes leads to an endless wait, which destabilizes the cognitive system and affects the ability to remember, to reason, to calculate times and distances, and to understand time and space. This creates an uncertain social representation of the self and can even lead to one losing the perception of reality.

In order to maximize the efficacy of the autopsy as well as the identification process of human remains, it is necessary to perform a postmortem examination following Interpol recommendations. The incomplete collection of such data could represent a violation of human rights, because once the fate of a missing person has been determined to be death, all available means must be undertaken to ensure recovery of the body and any personal effects.

Greater emphasis needs to be placed on the role of the forensic odontologist and on their collaboration during the autopsy of unidentified cadavers, regardless of the circumstances. Of the 832 bodies, it appears that only 61 bodies received an odontological assessment with a proper odontogram charting. The failure to routinely employ odontologists in missing persons investigations may result in a reduction of additional findings which, together with other circumstantial evidence, could lead to a delay in positive identification and actually prolong the condition of suspended grief, a situation which needs a body, even if such a body is lifeless, in order to bring a ritualistic "end" to the relationship.

Missing Persons, Human Rights, Forensic Odontology

F6 Fatality Management of a Multi-Vehicle Accident

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After attending this presentation, attendees will gain insight in the management of an accident with multiple fatalities by a state disaster team, Florida Emergency Mortuary Operational Response System (FEMORS). The overall result was the expeditious identification of the victims utilizing the appropriate resource allocation and minimum miscalculations. The nimble response was the result of 10 years of training augmented by several incident activations, including two minor hurricanes.

This presentation will impact the forensic science community by illustrating the efficiency of a successful disaster mitigation team, despite its limited size.

FEMORS was established in 2002 as part of the Health Department's ESF-8 Division of Emergency Management. Since then, FEMORS has been alerted for four hurricanes and activated for two more during the busy 2004 – 2005 hurricane seasons. Over the last 10 years, the team has engaged in annual disaster training including bi-yearly mock disasters. One of the important lessons learned from previous incidents is the importance of utilizing an assessment team deployed in advance to evaluate the true needs of the medical examiner. For example, initial estimates of deaths for both hurricanes Charley and Ivan were 20 – 60. These predictions were grossly inaccurate and, in the case of Hurricane Charley, led to a misallocation of valuable resources. Attendees will see in this case that the decisions of the assessment team and incident commander led to an efficient activation of only those resources actually needed for the incident. The entire operation was completed in four days utilizing limited personnel and resources with a total cost of approximately $20,000.

The Incident: Interstate I-75 Accident, Paynes Prairie, Florida, January 29, 2012. At about 4:00 a.m. on I-75 in Alachua County, just south of Gainesville, Florida, dense smoke from a brush fire along with fog rendered a stretch of the highway completely impenetrable.

Two major crashes occurred within minutes of each other due to vehicles stopping on the roadway. The northbound crash involved eight vehicles resulting in seven deaths and the southbound accident resulted in another four deaths and fire. Initially, there were thought to be 20 deaths and counting. In addition, there were dozens of victims taken to several hospitals with various injuries.

By 6:00 a.m., the Florida Highway Patrol (FHP) notified the Medical Examiner's (ME's) office of the accident and by 6:15 a.m., the Commander of FEMORS was also notified. FEMORS was activated for deployment a little more than an hour later. By 10:00, the entire designated team of 16 FEMORS members, including the assessment team, investigators, VIC personnel, forensic odontologists, and

command staff, were either already on the scene or on their way from various locations in Florida to the command center at the ME's office.

Two of the charred vehicles with occupants were moved to the Sheriff's office for collection of remains by 3:00 p.m. and the VIC center was conducting interviews with relatives by 4:00 p.m. By mid-afternoon the next day, Monday, five of the victims had been positively identified with two more presumptively identified. On Tuesday, 15 of the 16 individuals on all the missing person reports were accounted for, with one being one of the victims. Tuesday was devoted to careful extraction of burned, fragmented, and comingled victims from two vehicles by the forensic anthropologists and odontologists. One family had not been declared missing initially. Infrared (IR) examination of a charred document led to the names of the remaining individuals.

Finally, on Wednesday morning, the forensic odontologist identified the final victim, a 17-year-old female passenger. FEMORS was deactivated that day with a total of four days activation. Over the next several days, the medical examiner completed the final reports for identification.

Deficiencies were few and minor with the exception of the possibility of an incorrect identification by the hospital between two sisters of one family. Teamwork between the medical examiner and staff, FHP, and the FEMORS team led to an efficient recovery and identification of the victims.

Vehicular Accident, Disaster Response, Incident Response

F7 Mesio-Distal Width of Canines: A Tool for Sex Determination

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After attending this presentation, attendees will be able to determine the sex of an individual from a canine tooth in cases of bodies found as fragments or skeletal remains.

This presentation will impact the forensic science community by imparting a new tool in the determination of sex from a tooth.

Teeth, in the living as well as the dead, are the most useful objects in the field of forensic investigation. Their ability to survive in situations like mass disasters makes them an important tool in victim identification.[1] Though the morphology and structure of a tooth is similar in both men and women, there are subtle variations that could be valuable in the determination of sex. "Sexual Dimorphism" refers to those differences in size, stature, and appearance between male and female and can be applied to dental identification since no two oral cavities are alike.[2] Variations in the dental size can give a clue about differences between the sexes. Many authors have measured the crowns of teeth in both men and women and found definite variations. Canines, that are reported to survive air crash and hurricane disasters, are perhaps the most stable teeth in the oral cavity because of the labio-lingual thickness of the crown and the root anchorage in the alveolar process of the jaws.[2-7] Measurement of mesio-distal width of the mandibular and maxillary canines provides good evidence of sex identification due to dimorphism.[5]

Materials for the present study consisted of 500 students belonging to various parts of Karnataka, India, in the age group of 15 – 25 years comprised of 250 males and 250 females. The mesio-distal crown width of mandibular and maxillary canine teeth, i.e., the greatest mesio-distal width of the crown between the contact points of the teeth on either side of the jaw, was measured. The results obtained were subjected for analysis to derive conclusions. Sexual dimorphism in the right and left mandibular and maxillary canines was calculated using *Garn & Lens (1967)* formula.[6] The data obtained were quantified and analyzed statistically using a stastistics software package to determine the significance of differences between the sexes.

It was observed that the mean value of the mesio-distal crown width of right and left mandibular canines were higher in males than in females with statistical significance ($p < 0.001$). Similarly, the mean value of mesio-distal crown width of right and left maxillary canines was also more in males than in females with statistically significant value ($p < 0.001$)

The conclusions drawn were:

- Mesio-distal width of canines in all the quadrants for a given gender did not show any significant variation.
- The mean mesio-distal width of mandibular canines was greater in males than females.
- The mean mesio-distal width of maxillary canines was greater among males than females.

The results of the mesio-distal width of canines obtained could be of importance to anthropologists and of help to the clinical orthodontist for space assessment. It even helps forensic experts in cases of mass disasters in assessing the sex of an unknown remain.

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Mesio-Distal Width, Canine Tooth, Sex Determination

F8 Critical Effects of Improper Exposure of Digital Dental Radiographs Derived From PSP Plates

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After attending this presentation, attendees will understand the potential for erroneous dental X-ray image orientation (right vs. left) resulting in reversed (mirrored) radiographic data when images are improperly obtained using digital X-ray systems based on some Photostimulated Luminescence (PSL) systems.

This presentation will impact the forensic science community by demonstrating the results observed when properly and improperly placed image receptors are exposed on an X-ray phantom using both the traditional film-based systems compared to the emerging technology used in digital dental radiography. An understanding of the results presented will provide information critical to the accurate victim identification through dental findings by helping to prevent errors.

Dental radiographs, whether film-based or digital, may be viewed from either the "front" or "back." Thus, if an observer evaluates the radiographic image from a viewpoint opposite from the image surface which was exposed, the determination of whether the structures seen are from the subject's right or left will be incorrect. This means, for example, that what may be perceived as the maxillary right first molar (tooth #3) in actuality is the first molar on the left side (tooth #14).

Any putative dental identification must be ruled out if any unexplainable disparities are noted between the antemortem and postmortem dental findings. Incorrectly orienting or viewing image media can erroneously create such disparities and prevent an accurate dental identification. Dental film manufacturers have traditionally placed a palpable "dimple" or "bump" on every film. This can be used to assure that the image is properly oriented when viewed. Additionally, a metal foil backing with a distinct pattern is included in the film packet behind the film to warn the viewer if the film was exposed from the improper side (from the non-exposure or "back" side).[1]

Typically, duplicated antemortem radiographic series do not provide the aforementioned physical bump for the examiner as a reference. This has been shown to be particularly troublesome when there are a very large number of decedents and when many of the antemortem images submitted are duplicated rather than original radiographs. There are several ways to determine the film's original orientation but most forensic odontologists accomplish this using the spatial location of the bump within the image itself. When the bump appears at either the lower right or upper left corner of the image, this assures that the dental structures are being viewed as if the viewer is facing the dental arches (bump-up). Bumps in the upper right or lower left corners of the images mean that the structures are being observed from the vantage point of the tongue (bump-down).[2,3] Also, panoramic images are typically labeled with "left" and "right" indicators in the image using letters either placed within the film cassette or on the device's head-holders.

However, there is a great potential for inadvertent spatial errors when interpreting improperly placed dental X-ray images captured by the Air Techniques ScanX™ Phosphor Storage Plate (PSP) system. PSP technology is considered an indirect digital radiographic system in which plates coated with photostimulable phosphor capture and store X-ray energy when images are taken. The exposed plates are then passed (scanned) through a laser which releases the stored energy in the phosphor as light. The light released varies according to the amount of stored energy, thus producing a radiographic image. The failure in the system arises from the fact that a plate which has been improperly exposed from the non-exposure side produces a "flipped" image with no indication after image processing that a spatial error has occurred. This is because the plates have no reference to indicate errors in that they lack the lead foils found in film packets. Also, the design of placing a reference letter "a" on the exposure surface of the plate is flawed and is not indicative of orientation as are the "bumps" in specified film corners previously mentioned. Additionally, this possible confusion concerning "left-and-right" orientation may occur with both intraoral and panoramic PSP images.

In conclusion, it is impossible to determine from a single digital radiograph if the portrayal of the decedent or victim's left or right is correct when created using the Air Techniques ScanX™ PSP system.

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PSP Dental X-Ray, X-Ray Errors, Dental Identification

F9 Dental Identification Without Antemortem Dental Records: Case Example From Central America

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After attending this presentation, attendees will understand an application of "smile line" dental identification in a murder case in Central America. Dental identification without antemortem records is certainly problematic. Yet there are a number of circumstances where this scenario might occur: cold cases, mass graves, gang-related murders, drug violence, civil war, fraud, misidentification, etc. DNA recovery may not be an option leaving few other options for identification.

Central America is plagued by a prolonged history of civil war, drug violence, and femicide. According to the United Nations, the homicide rate in Central America has reached a crisis point with the Honduras homicide rate at 82/100,000, El Salvador with 66/100,000, Mexico at 18/100,000, as compared to the U.S. with 5/100,000. This presentation will impact the forensic science community by showing how dental identity can be accomplished without antemortem records in limited situations.

Not surprisingly those countries with the highest overall rates of violent crime have the highest rates of femicide as well. The pervasive culture in Central America is that women are easily disposed of with impunity. El Salvador leads this metric with 12/100,000 female population with Guatemala third at 9.7/100,000. The violent nature of these crimes has resulted in countless unidentified victims and grieving families.

Dental identities by traditional comparative methods often are not possible. Typically, there is a lack of dental history as a consequence of poverty or intentional concealment because of illegal activities. Yet with the ubiquitous use of cell phones with cameras, a creative approach to dental identification may be applicable. This "smile line" concept of identification is not new in the odontology armamentarium. However, participation in a human rights symposium for women in Central America revealed a total lack of awareness of the application of this methodology for identification. The participants overwhelmingly expressed an interest in this simple, inexpensive, fairly effective modality when identifications are an issue.

Case in point, a forensic team consisting of two forensic anthropologists, a medical examiner, a forensic odontologist, and support staff were tasked with determining the cause and manner of death and a dental ID for a young woman believed to have been murdered in a Central American country.

The court granted permission for an exhumation and autopsy to be done with the resident pathologist. The forensic team was sent from the U.S. to work with the local officials. The victim was exhumed and transported to the morgue where a thorough autopsy was performed on the decomposed remains including a complete dental examination with digital images. Based on the quality of the dental restorations and the fact that the family resided in the U.S. for several years, there was a presumption of available dental records. None were discoverable. A visual ID was not possible due to severe decomposition but a presumptive ID was originally done based on artifacts accompanying the body. The family provided several photographs of the decedent showing the anterior teeth. The photos were imported into Adobe® Photoshop® for appropriate enhancements. The photos were of fair quality but somewhat grainy which led to pixilation when enlarged. Visual examination of the victim revealed a number of individual dental characteristics including rotated and tilted teeth, several amalgam restorations, and incisal irregularities. The photos provided a dental profile sufficient to compare to the dentition of the victim. The cause of death was determined to be homicide, the manner to be strangulation, and a positive identification was made.

Beyond the relevance of just this individual case, the utility of using images from a digital camera or a cell phone camera as a source of antemortem images for potential dental identifications in situations where there are limited or no dental records is beneficial.

Dental Identification, Smile Line ID, Murder Case

F10 The History of Tattoos and Their Use as a Means of Identification

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After attending this presentation, attendees will have a better knowledge of the development of tattoos and how they can be used as a means of identification.

This presentation will impact the forensic science community by demonstrating how tattoos can be used as an identifier.

As one can imagine, the art of tattooing was been around for quite some time. In fact, there is evidence to demonstrate that tattooing was done thousands of years ago.

One of the oldest known specimens is that of Otzi the Iceman, dated around 3300 B.C. He had 57 separate tattoos. Among these were a cross on the inside of the left knee, six straight lines, fifteen centimeters long above the kidneys, and numerous small parallel lines thought to be possible therapeutic tattoos for the treatment of arthritis. There have also been several mummies from West China as well as mummies from permafrost dated around 300 B.C. It is obvious, therefore, that tattooing has been around for quite some time. In fact, evidence shows that tattooing was quite prevalent in China as well as Egypt, India, and the Philippines, just to name a few. Further, in the Philippines, tattooing to some was a form of rank and accomplishment. Some believed that tattoos had magical qualities. Many tribes have a tattoo culture, such as those found in Indonesia.

In Europe, pre-Christian Germanic, Celtic, and other central and northern European tribes were often heavily tattooed. During the gradual process of Christianization in Europe, tattoos were often considered remaining elements of paganism and were, for the most part, legally prohibited.

As previously mentioned, tattooing was exhibited in China as early as 3300 B.C.; however, recently, tattooing for spiritual and decorative purposes in Japan is thought to extend back to at least the Paleolithic period. This would be about 10,000 B.C.

In Samoa, the traditional male tattoo is called the pe'a and the traditional female tattoo is called the malu. The word tattoo is believed to have originated from the Samoan word "tattoo."

Tattooing has obviously been a practice for thousands of years as evidenced in many cultures although the reasons vary greatly because of culture, religious beliefs, and society. Some consider tattooing an art form, while others consider it a ritual or requirement to have performed. During the latter decades of the 20th-century, tattooing became a popular social practice worldwide. Many younger (and some older) people today either have aspirations to have a tattoo somewhere on their body, or already have one or more. As we all know, the reasons vary as well as the shape and size of the tattoos.

Some cultures and religions ban tattoos. The orthodox Jews, for example, abide strictly to the third chapter of Leviticus, which states that one should not make gashes in one's skin for the dead: "Do not make marks on one's skin." Modern tattooing is included in this belief and is, therefore, not permitted. The Christian churches do not prohibit tattooing so long as the tattoo is not an image directly opposed to Christian teaching or religious sentiment. As with the Jews, the Muslims also forbid tattooing.

It is therefore observed that tattoos are obtained for a variety of reasons. Further, tattoos can obviously be used as an identifier of an individual. Actually, tattoos can identify an individual in one of two ways. First, at times, an individual may have a tattoo as a requirement or a symbol of a group he or she belongs to, such as a gang. Perhaps someone is tattooed against one's will, as in the case of a prison camp. Secondly, a tattoo can simply be used to identify a person because of the fact that someone else is aware of the tattoo and the location on the body of the tattoo.

In conclusion, whatever the reason, tattoos have been around for thousands of years. And, tattoos most likely will be around for many thousands of years in the future. Of the many ways to identify an individual, tattoos are simply another way to acquire a positive identification.

Tattoos, Skin Markings, Identifying Tattoos

F11 The Case of the Missing Molar

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After attending this presentation, attendees will understand how identification of an unidentified decedent by using dental records is not always straightforward, and that it is imperative to carefully review the antemortem and postmortem dental records. Dental identifications are generally thought of as being a somewhat routine forensic odontology procedure, but presented here is a case with a very interesting "twist." This case has interesting ramifications for both dental identification and dental age estimation procedures.

This presentation will impact the forensic science community by demonstrating that the accepted axioms in a field may not always hold true in every case. For example, this study contends one of the well-accepted foundation beliefs in the theory of dental identification is that, in the permanent dentition, if a particular tooth is *missing* in the antemortem records, but is *present* in the postmortem records, there is an unexplainable inconsistency that indicates an exclusion. But this case will demonstrate that this idea may not always be true.

In 1995, the skeletal remains of a young woman were found near Houston, Texas. Virtually the entire skeleton was recovered, including the cranium and the mandible, both with intact dental arches. The skeleton was sent to an anthropology laboratory for examination and analysis. The anthropologist charted the condition of the teeth and the dental restorations present. The anthropologist compared these postmortem dental records to the antemortem dental records of a young female (JC) who was missing in the Houston area. The anthropologist concluded that, while there were many "similarities between the dental records of JC and the dentition of the unidentified skeletal remains," there were two dental inconsistencies that indicated the remains were probably not those of JC. He concluded "although this exclusionary trait casts doubt on the remains being JC, I suggest that it be evaluated by a forensic odontologist for confirmation." Regrettably, this suggestion was not followed until 2012 when the written dental records were submitted for review in July of 2012. It was noted the exclusionary factors which had been documented by the anthropologist, but urged the investigating agency to obtain the skeletal remains and original dental records for examination. The records were sent for review and a second opinion, and it was agreed that the original dental records and the remains should be obtained for examination before a definite conclusion could be reached.

In 2008, the skeletonized remains had been submitted to a DNA lab for examination and analysis. In early August of 2012, a DNA profile was extracted and the analysis indicated it was 4.4 trillion times more likely that the remains were those of 20-year-old JC rather than a random female from the Caucasian population. The decedent's remains and the dental records of JC were hand-delivered by the investigating agency in late August of 2012.

One of the potential exclusionary factors, a tooth noted in the antemortem dental radiographs to have a restoration while the corresponding tooth in the remains had been charted as unrestored, was immediately resolved upon examination of the actual remains. Now the "twist:" tooth #32 (Universal) was clearly missing in several antemortem dental panoramic films (the other third molars were present and unerupted), and, on visual inspection, tooth #32 also appeared to be missing from the remain's mandible. However, postmortem radiographs of the mandible showed #32 was clearly present but unerupted. The DNA analysis said "match," the dental records said "exclusion." The attendees viewing this presentation will learn how the case was resolved.

Dental ID, Age Estimation, Exclusion

F12 Forensic Dental Analysis of Degraded, Fragmented, and Commingled Human Remains

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After attending this presentation, attendees will recognize the morphological, anatomical, and pathological criteria used in forensic dentistry and its application as an important tool in identifying commingled remains of skeletonized bodies in a mass grave intentionally disturbed.

This presentation will impact the forensic science community by demonstrating how an accurate forensic dental analysis of degraded, fragmented, and commingled human remains can be essential for

individual identification even when antemortem records are not available.

During the military regime in Chile, thousands of people were killed. In many cases, they were caught and killed in groups and illegally buried in mass grave sites. After a period of five to ten years, when some of the graves were discovered, the military carried out an operation to remove the bodies from the mass graves to make evidence disappear. At that time, bodies were skeletonized so they could not remove all the remains, leaving in place bone fragments, teeth, and other small evidence. With democracy, judicial investigation of these cases started and the state forensic institution "Servicio Medico Legal" (SML) was called to perform exhumations of remains in different disturbed burial sites.

This particular case involves a group of farmers killed in a creek and buried in a mass grave that was disturbed from remotion five years later. Despite this, the SML was able to recover human bone fragments, dental remains, ballistic evidence, personal objects, and non-human skeletal remains, all commingled, incomplete, and degraded.

Forensic dentistry is a useful tool in human identification, which achieves its goal through the comparison of antemortem and postmortem information. The postmortem examination is affected in this case by the admixture of remains as well as postmortem tooth loss caused by the intentional relocation process. Additional difficulties derived from the long interim from burial to judicial exhumation (30 years). Performing a thorough dental macroscopic study using morphological, anatomical, and pathological criteria, evidence was organized in skeletal and dental clusters, partially or completely reconstructing the dental arches and providing the minimum number of individuals present in the mass grave.

One hundred and twenty-seven human remains good for dental analysis were recovered: 111 (87%) consisted of isolated teeth (including crown fragments), ten (8%) were mandibular fragments (four with *in situ* teeth), five (4%) were maxillary fragments (three with *in situ* teeth), and one consisted of a removable upper dental prosthesis. All specimens presented taphonomic erosion with the teeth being the least affected due to dental enamel resistance.

Odontological analysis classified 87 of 127 specimens into 21 groups obtaining two complete upper arches, one complete lower arch, two partial upper arches and one partial lower arch, and other smaller parts. This led to a minimum of nine individuals for the burial site, considering only the odontological point of view.

The lack of adequate antemortem dental records ruled out postmortem comparison for identification purposes, thus genetic testing appeared as the only possibility to get individual identities. Teeth from some of these twenty-one groups and eight ungrouped teeth were subjected to nuclear DNA sampling; one to three samples from each group were sent separately. A consistency up to 100% was obtained between odontological analysis and DNA testing: identical genetic profiles in all loci typed for teeth coming from the same group. The genetic testing results confirmed the odontological clusters and also provided other associations between upper and lower arcades and ungrouped teeth.

The dental analysis methodology and evidence organization allowed grouping and adequate selection of samples for genetic analysis that led to successfull identification of individuals, preserving dental remains suitable to be returned individually to relatives, and terminate the grieving process. Multidisciplinary approach integrating judicial information, forensic archaeology, forensic anthropology, forensic odontology, forensic pathology, and forensic genetics was essential for this goal.

Commingled Remains, Odontology, Identification

F13 The Characteristics of Dental Restorations in Korea Before 1970: To Differentiate Between American and Korean Remains From the Korean War

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After attending this presentation, attendees will be able to better identify remains of Koreans from those of American soldiers lost in the Korean War through understanding the characteristics of Korean prosthetic restorations performed before 1970.

This presentation will impact the forensic science community by providing information on the distinctiveness that can identify Koreans from commingled remains – even those found in other countries due to the different conflicts, mass disaster, or aircraft crashes which occurred in the 20th-century.

Dental prosthetic restorations artificially substitute for lost teeth. The first evidence of the dental prosthetics was the gold wire ties used for fixing neighboring teeth in Egypt around 2500 BC. Afterward, the dental prosthetics have developed tremendously. In the 18th-century, French surgeon Pierre Fauchard, credited as the "Father of Modern Dentistry," published the fundamental dental book which was the advent of modern dentistry. In Korea, modern Western dentistry was first introduced in early 1900s. The first dental school, Department of Dentistry in Severance Union Medical College, opened in 1915 while the first dental college, Baltimore College of Dental Surgery, was established in 1863 in the United States of America. The emergence of Korean dentistry was delayed due to the late introduction of modern dentistry during the Japanese occupation (1910 – 1945) and the Korean War (1950 – 1953). The imperialist Japan even licensed the dental technicians and let them practice dentistry freely. It is speculated that such historic background might be one reason for the unique prosthetic restorations in Korea at that time. The purpose of this presentation is to investigate the characteristics of Korean prosthetic restorations performed before 1970.

This study used 74 prosthetic restorations from 57 remains of Korean servicemen excavated from Korea in 2009. These dental remains are believed to be Koreans because they shared similar and unique restorative patterns. Forty-two remains were estimated to be male with 15 having no sex determination. Thirty-six remains were estimated to be young adults (17 – 25 years), four middle-age adults (26 – 40 years), and 17 undetermined.

Single crown and crown and bridge comprised 48 and 26 respectively out of 74 prosthetic restorations. Among crown and bridges, 18 restorations had one or more missing teeth in that structure, but eight restorations didn't involve any missing tooth. An interesting feature is that most of these crowns were oversized compared to normal teeth. Totally, 124 crowns including single crowns (48), abutments (55), and pontics (21) were observed. Fifty-seven were full crowns, 55 were ¾ crowns with open face on the facial surface, and 12 were wrapped crowns with open face at occlusal surface. It is assumed that most of these restorations were probably fabricated not by a casting method but by a sealing-and-forging method because the teeth were barely prepared. Therefore, it was found that many crowns were open at the occlusal, lingual, and facial surface. It is also speculated that even full crowns had very thin occlusal and incisal surface because of the lack of tooth preparation.

Out of 74 prosthetics, 56 restorations (75.7%) were found at the maxillary anterior teeth area while nine (12.2%) were found at the mandibular anterior teeth, six (8.1%) were at the mandibular posterior teeth, and three restorations (4.0%) were at the maxillary posterior teeth area. It is interesting that most restorations on the maxillary anterior teeth area included maxillary lateral incisors (43/56, 76.8%) which were

not missing. According to the traditional Korean beliefs, good luck "leaks" through the space between the teeth. In order to prevent this, soldering rest or big-sized crowns were fabricated. Also, there were no esthetic considerations choosing materials for most of the restorations. For example, cheap metal materials (56/74, 75.7%) were used most often, even for the anterior teeth (87.8%). Some restorations were made of gold (8/74, 10.8%) and stainless steel (10/74, 13.5%).

The characteristics of Korean dental restorations fabricated before 1970 can be summarized as follows. First, crowns have an enlarged shape, probably in order to close the space between teeth. Secondly, restorations are opened or very thin at the occlusal and facial surface due to the difficulties of tooth preparation. Lastly, there is little esthetic consideration for the anterior teeth. These characteristics might help identify Koreans from commingled remains of past wars or disasters.

Prosthetic Dental Restoration, Korean Identification, Forensic Odontology

F14 A Brief History of Forensic Odontology in Turkey

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After attending this presentation, attendees will learn about the development of forensic odontology in Turkey.

This presentation will impact the forensic science community by describing different odontological cases in Turkey.

Though being a very important discipline of forensic sciences, the scientific use of forensic odontology as a forensic field in Turkey is new, starting in 1994. The first case was a homicide case with a bitemark and the bitemark was successfully identified; this finding was accepted as evidence by the court. After this, papers on the application of dentistry in forensic cases emerged and increased in Turkish forensic journals. This presentation attempts to summarize the history of forensic dentistry which gained its important role in forensic cases very late in Turkey with sample applications carried out in the Council of Forensic Medicine of Turkey.

Samples of forensic odontology cases in Turkey in a chronological manner:

1993 – A Skeletonized Human Corpse Found in Antalya: A skeletonized corpse was found in the hilly region of Antalya together with his bags. There was information of a missing German tourist who was interested in mountain climbing. Skeletal remains of the case and personal belongings were sent to the Council of Forensic Medicine for identification. The skeletal remains were examined by a forensic pathologist and forensic odontologist and comparison of dental restorations with antemortem dental records resulting in positive identification of the case.

1994 – Bitemark Analysis in Elmali, Antalya: In a homicide case, the photograph of a bitemark on the left humeral region of the victim and a dental cast of the suspect were sent to the Council of Forensic Medicine for comparison. After examination, it was reported that dental characteristics of the bitemark and the dental cast of the suspect were the same.

2003 – A Serial Killer in Istanbul: A serial killer created terror in Istanbul and drew the attention of both the public and press. The victims of the killer were older woman living alone. With the first victim, there were many bitemarks on the arms, face, and buttocks. The next victim was killed by stab wounds. There were bitemarks on this woman as well. One and a half months later, he assaulted an elderly American woman living alone in the same region. After careful examination of bitemarks and comparisons with the dental casts of the suspects, the serial attacker was successfully identified.

2003 – Success in International Works: As a part of the ABFO workshop in Chicago of 2003, photographs of the bitemarks and dental casts of three suspects were sent to eighteen experts for comparison. Using five methods, comparisons were performed, results presented, and the cases were successfully identified.

2003 – Mass Disasters in Diyarbakır: A Turkish RJ 100-type aircraft crashed at the military airport that is also used for civilian flights. Only five persons out of 85 survived. The remaining 72 adults, three babies, and five flight personnel were dead. Despite the presence of the burns (almost to the degree of charring), identification of 55 victims was possible. The identification of five of the remaining 20 victims was carried out by the DVI team from the Council of Forensic Medicine in Istanbul by comparing antemortem and postmortem dental records and photographs.

Historical Cases: Historical skeletal remains were found in Maiden's Castle during an archaeologic dig. Kiz Kalesi (Maiden's Castle) is located on a small island approximately 60km from the Mediterranean city of Mersin. It was reportedly built in the 12th-century AD. During its restoration, archaeologists unexpectedly discovered human remains. After excavation, these remains were sent to the State Institute of Forensic Medicine in Istanbul for dental and cranial examination. V-shaped dental mutilation and craniofacial mutilation were identified in two of the skeletons. These cultural custom V-shaped dental mutilations matched Romero Dental classification A1.

Odontology, History, Identification

F15 Assessment of Age by Teeth

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After attending this presentation, attendees will be able to understand the methodology for estimating the dental age-aided design software and layout of data and results for how adults and elderly victims died.

This presentation will impact the forensic science community by showing how computerized methodology of age assessment provides reliable ergonomics and how its use in laboratories or mobile laboratories is important. This methodology is currently being tested in the Forensic Science Institute of French Gendarmerie (I.R.C.G.N). The practical applications presented can be used as a guideline available to forensic odontologists.

Each method of age assessment has advantages and disadvantages. The search for precision includes many factors (conditions of nutrition, life, growth, normal or premature aging, and disease). Currently, forensic practices are moving toward a dual assessment of age in two different systems, the evaluation of bone age and dental age (Pasquier 1999).

Age assessment is an important element of dental forensic examination. It is often required by magistrates and investigators as part of the dental analysis. Different methods exist to determine the dental age with the most appropriate depending on the case to be examined. The choice of method is multifactorial and depends not only on the categorical age of the victim (child, adolescent, adult, elderly), but also on the status of the victim (living or dead), and finally the technical constraints.

The goal of this work is to develop a suitable methodology for assessing age in deceased adults and elderly victims. This methodology involves the use of two methods, taking into account the histological and physiological factors. The Lamendin Method (1990) uses the entire tooth without preparation and studies the root transparency (root dentin sclerosis) and periodontosis factors. The Johanson Method (1971), derived from the Gustafson Method (1947), takes into account attrition, secondary dentin, periodontosis, cement apposition, root resorption, and root transparency. A value is assigned to each factor correlated with age. Each factor has seven levels of interpretation. Using two methods tends to confirm the probabilities when the results are consistent. Otherwise, it is necessary to seek the cause of inconsistent results.

A second step consists of automating data collection and calculation of age for both methods. Regarding the Lamendin method, the teeth selected for the evaluation are photographed on a light box. A camera connected to a computer allows shooting with remote target monitor. This photograph is included in the processing software. Measurements of the height of the root, the heights of periodontosis, and root transparency are made, then the estimated age is automatically calculated. On the Johanson method, a fine cut is performed with a microtome equipped with a diamond saw. This cut is photographed and six factors are measured. The results are automatically plotted on a summary explanation.

The use of different dental methods of estimation is interesting. Estimation applied to several teeth of the same victim is recommended. Consistency of results is essential; if there are significant differences, the cause must be investigated. The use of photography and processing software allows reliable measurements, observations, and facilitated automated results. In the end, the system provides significant time savings.

Dental Age Assessment, Lamendin Method, Johanson Method

F16 Correlation Between Biological and Chronological Age of Bodies in Colombia — Exhumed and Identified

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After attending this presentation, attendees will understand the reasons why there is a need for studies of skeletal remains in samples of Colombian population.

This presentation will impact the forensic science community by showing how some of the methods that are currently applied in Colombia for the estimation of biological age in skeletal remains are not adequatefor such a purpose considering that most of the methods were supported from samples of foreign populations.

In Colombia, there had not been a study to establish the correlation between biological age estimated by skeletal remains using both dental and anthropological analysis methods with the chronological age of the same provided by the National Registry of Civil Status in bodies exhumed and identified from 2005. Age is a key variable for the process of identifying bodies and searching for missing persons; it is important to note that most of the methods used for estimation of biological age in bony debris were performed on samples of foreign populations and only some of these methods have been validated for Colombian populations. In Colombia, there exists a high number of human rights violations. Through the years, dozens of people have disappeared or died due to armed conflict and has resulted in buried and unidentified corpses in unmarked graves, morgues, and cemeteries, which has led to state agencies actively promoting the process of identification. In the course of daily practice, forensic experts often analyze different cases of skeletal remains exhumed somewhere in the country. This is why it is important that estimated biological age in skeletal remains is as close to chronological age of the victim so that the identification process is properly oriented and thus reducing the number of unidentified bodies.

Objective: To determine the correlation between biological age, estimated in skeletal remains, by two analysis methods (dental and anthropological) with chronological age.

Materials and Methods: The study was based on the traditional positive scientific method of investigation and was framed in descriptive research with a non-experimental design. The sample consisted of 123 cases of bodies exhumed, identified, and returned to their families, who were selected at random from a total of 1,070 cases. The determination of the sample and the analysis of the study were conducted with the implementation of Estata 10. Descriptive analysis was made of the information unifying age variables, obtaining averages, and standard deviations. To evaluate, the correlation coefficient used Lin's concordance and correlation with their respective ranges of 95% confidence. The study was approved by the research and ethics committee of the Faculty of Dentistry, Pontificia Universidad Javeriana Bogota-Colombia. Throughout the implementation and application of the results, there was no effect, positive or negative, in the short, medium, and long term, on the environment and natural human health.

Results: A coefficient value was obtained for anthropological analysis Lin (0.782) and dental analysis (0.765).

Conclusions: This research demonstrated, according to Lin, a degree of good concordance; however, there are cases that conclude that some methods are not reliable for the Colombian population. In such cases, to use the biological age as a parameter, identification would exclude a group of bodies in the process of tracing missing persons. Forensic experts apply established methods for estimating biological age. When cases come forward with a wide age difference with the concordance perfect line, this indicates that the proficiency of the forensic experts is good; however, some methods that are currently applied in Colombia for estimating biological age are not suitable for this purpose, considering that most methods were supported in samples of foreign populations.

Studies are recommended in bony debris of Colombian population samples in order to endorse all the methods currently applied for the estimation of biological age.

Biological Age, Chronological Age, Skeletal Remains

F17 Human Third Molars Development: Comparison of 13 Country Specific Populations

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After attending this presentation, attendees will be informed on the detected differences in third molar development comparing 13 country specific population samples.

This presentation will impact the forensic science community by increasing awareness on how the comparisons of third molar development between individuals from different countries revealed distinctions in speed and onset of development, but although reaching statistical significance, these differences were small and not constant over the considered age range.

Dental age estimations in the sub-adult age group are mainly based on the development of the third molar(s). In forensic practice, these age estimations are primarily requested to advise legal authorities in their judgments related to the age of unaccompanied young asylum seekers. Although the majority of age estimation models based on third molar development are constructed on reference samples with ethnically and/or geographically well-described and outlined origin, these studies can rarely be compared for the evaluation of possible ethnical or geographical influences on third molar development.

In an ongoing data collection, previously nine country specific samples (Belgium, China, Japan, Korea, Poland, Thailand, Turkey, Saudi Arabia, and South India) were investigated on third molar development. In the present study, four new country specific datasets (Brazil, Italy, Malaysia, and United Arab Emirates) were added, analyzed, and compared. The goal of this study is to collect country specific databases of third molar development and to evaluate and compare third molar development between the collected countries.

A total of 10,117 panoramic radiographs from subjects out of the previously listed 13 countries were collected. For each country, the individuals were homogeneously distributed in the age range between 16 and 22 years and in sex. Third molar development of all present third molars was registered, according to the ten stages technique of Gleiser and Hunt, modified by Köhler.[1,2] Missing third molars received a zero score. Consequently, each subject received a third molar score sequence for the upper right, upper left, lower left, and lower right third molar, respectively. To obtain a factor score for each subject,

representing the degree of third molar development in the total dataset, a generalized linear mixed model for multivariate ordinal data was fitted on the third molar score sequences of all subjects from the 13 countries. Differences in degree of third molar development between countries were analyzed using gender-specific regression models for these factor scores with age and country as predictors.

Comparisons between countries revealed differences in speed and onset of third molar development. Among the different ages, the degree of third molar development changed between countries in an unordered way. No clear patterns of differences in degree of third molar development could be distinguished between the countries. Compared to all other countries, Belgium subjects were generally developing fastest. As such, using for age estimations purposes, third molar development information from Belgium instead of country specific information will result in underestimated age predictions. This is the best judicial reference if country specific reference information is lacking. Indeed, legally speaking, an advantage is then provided of the doubt for the individual under examination.

In conclusion, no evidence was detected for important differences in degree of third molar development between the 13 examined countries. This implicates that geographical differences between examined individuals are of minor influence on the age predictions based on third molars development.

References:

1. Gleiser I, Hunt E. The permanent mandibular first molar: its calcification, eruption and decay. *Am J Phys Anthropol* 1955;13:253-83.
2. Köhler S, Schmelzle R, Loitz C, Püschel K. Development of wisdom teeth as a criterion of age determination. *Ann Anat* 1994;176:339-45.

Age Determination, Third Molar Development, Country Specific Population

F18 Optimal Dental Age Estimation Practice in United Arab Emirates (UAE) Children

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After attending this presentation, attendees will understand how there is a need to develop a United Arab Emirates (UAE) reference model, or that the Willems model can be used to perform dental age estimations in UAE children.

This presentation will impact the forensic science community by imparting knowledge that verifies the Willems model on a UAE sample and comparing the outcomes with the verification of a UAE specific model revealing no or negligible differences.

Willems et al. dental age estimation method for children was developed on a Belgian reference dataset of 2,523 subjects.[1] The goal for this study was to detect if this method could be used for age estimations on UAE children or if it should be modified using a large UAE reference dataset. Furthermore, verification was needed to determine if adding third molars development information to permanent teeth development information in children provides more accurate age predictions.

Retrospectively, panoramic radiographs of 1,900 children with UAE origin and nationality were collected (950 female, 950 male). The selected individuals were homogeneously distributed in the age range between four and twenty-three years. All included individuals who had no medical history and no obvious dental pathology affecting the development of permanent teeth or third molars. The development of left mandibular permanent teeth was registered according to the eight-stage technique described by Demirjian et al.[2] The third molars development was registered using the ten-stage technique developed by Gleiser et al. and modified by Köhler et al.[3,4] The registrations of the tooth development was tested with Kappa statistics on inter and intra observer reliability after rescoring 100 randomly selected panoramic radiographs. The obtained permanent teeth data were used to validate the Willems et al. method. Next, these data were randomly, but stratified on age and sex, divided in a reference and a test dataset. The reference dataset was used to develop a UAE specific model according to the Willems et al. method. The established UAE specific model was verified using the test dataset. Multiple regression models with the scores of permanent tooth development, third molar development, and permanent tooth and third molar development as an independent and age as a dependent factor were developed. These models allowed detecting if adding in child third molar development to permanent tooth development, providing more accurate age predictions.

Almost perfect agreement was detected for intra and inter observer agreement (Kappa >0.86). Verifying the Willems et al. method on the UAE children dataset provided a difference in mean chronological age minus mean predicted age for males and females combined of -0.01 year (overestimation). The verification of the Willems et al. method compared to the verification of the UAE specific model revealed differences in: mean error of -0.12 (F) and 0.12 (M) year, mean absolute error of -0.07 (F) and 0.02 (M) year, and root mean squared errors of 0.08 (F) and 0.01 (M) year. The accuracy of age prediction adding third molar development to permanent teeth development was expressed in an increase of root mean squared errors of 0.02 years for males and a decrease in root mean squared errors of 0.02 years for females.

The results indicate that the Willems et al. model developed on a Belgian reference dataset can be used with negligible error for dental age estimations in UAE children. Adding third molars development information to the permanent teeth information in UAE children does not provide an overall gain in accuracy of the age predictions.

References:

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2. Demirjian A, Goldstein H, Tanner JM. A new system of dental age assessment. Hum Biol 1973;45:211-27
3. Gleiser I, Hunt E. The permanent mandibular first molar: its calcification, eruption and decay. Am J Phys Anthropol1955;13:253-83.
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Age Determination, Willems Method, United Arab Emirates

F19 Cementochronology: Improvement of the "Tooth Cementum Annulations" Method for Age Estimation

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After attending this presentation, attendees will understand and appreciate how the Tooth Cementum Annulation (TCA) method can provide accurate results for individual age estimation, which is one of the most crucial biological variables obtained during an osteological examination of human remains.

This presentation will impact the forensic science community by demonstrating how the TCA method improves the field of age assessment through a distinct physiological phenomenon.

Growth markers can be found both in bones and teeth, the latter being the most reliable due to their hard and compact composition. Within tooth tissues, acellular cementum begins its regular growth with tooth

eruption and then continues to be produced throughout as incremental layers of alternating dark and light bands of controversial origin. Cementochronology, or TCA, involves the counting of these incremental lines in tooth root acellular cementum using light and polarized microscopy.

Stott et al. (1982) were the first to precisely correlate the number of cementum annulations directly to calendar age in humans. Since then, many researchers published correlation rates above *r=0.9* effectively making cementochronology the most precise technique for individual skeletal age estimation.

In this study, 250 recent teeth from known age individuals, collected at the Lille Dental Surgery Department, were used. Within this sample, 50 teeth were specially extracted because of periodontal disease and 50 were selected from individuals above 65 years of age. The study focused on two main issues in order to improve the TCA method:

1. Correlation between estimated age and calendar age, with a specific attention to the influence of periodontal health and advanced age.
2. Better understanding of the histologic structure of the observed light and dark layers, using Raman Spectrometry.

In the first part of the study, all teeth were embedded in a two-component epoxy resin and dried in a vacuum chamber. Six sequential 100mm – 130mm undecalcified cross sections were prepared for each tooth, from the middle third of the root, with a precision saw, in order to be properly observed under light and polarized microscopy. QSegments that showed readable lines were captured as JPEG images and read with appropriate software. Three observers were involved in each counting process.

In the second part of the study, five finely polished, thick cross sections (700mm), essential to optimal exploitation of Raman spectrometry, were prepared.

The results showed a statistically significant (Spearman Test) strong correlation ($r > 0.88$ for all observers) between estimated age and calendar age. Inter observer errors were minimal between the three observers (Pearson's $r > 0.9$) with the highest correlations obtained from the most experienced author. Also demonstrated was the influence of periodontal disease and old age, which both decrease the accuracy of the technique.

The Raman spectrometry observations yielded promising preliminary results that seem to indicate a difference of orientation of hydroxyapatite crystals and collagen fibers rather than a difference in organic or mineral composition between incremental layers.

Odontology, Age Estimation, Tooth Cementum Annulations

F20 Updating a Dental School Forensic Odontology Curriculum in the Post 9/11 Era

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After attending this presentation, participants will be able to describe a predoctoral forensic odontology curriculum responsive to community needs post 9/11, develop a well-rounded curriculum in forensic odontology, and develop partnerships between a dental school and a regional medical examiner's office to offer student rotations to a medical examiner's office.

This presentation will impact the forensic science community by stimulating the interest of future dentists to the field of forensic odontology, providing graduating dental students with an understanding of the importance of record keeping and the role of their patient records in a victim identification effort, and developing a cadre of dentists who will have the necessary foundation knowledge to volunteer in a time of crisis.

This paper presents a course developed to reflect the current needs of the dental curriculum in a post 9/11 world and to help prepare dentists to serve their community.

The overall objective is to familiarize senior dental student with the various aspects of forensic dentistry including an overview of the role of the medical examiner, medical legal investigator, the forensic dental team, and the community dentist; as well as an introduction to victim identification based on dental records, bitemark analysis, photography for the forensic dentist, forensic anthropology, and forensic dental software. In addition, the course has been designed to provide students the foundational knowledge necessary to be a productive member of a mass fatality forensic identification team.

Curriculae include:

- **Introduction to Forensic Dentistry and Medicine**: This is a general overview of the field.
- **The Forensic Dental Team**: An explanation of the various components of the forensic dental team, roles of the various components of the team, and how to create a forensic dental team.
- **Dental Identification**: The importance of assisting in victim identification and what role the forensic dentist plays in the identification.
- **Bitemarks and the Role They Play in Criminal Investigations**: A discussion of past and present research into bitemark analysis and the uniqueness of the human dentition.
- **Mass Disasters**: The role of the dentist in assisting and making a dental identification during a mass fatality incident, i.e., antemortem, postmortem, comparison, and recovery teams.
- **Forensic Anthropology**: This lecture reviews the role of the forensic anthropologist in assisting in the identification of victims of a mass fatality incident as well as the unknown.
- **The Role of the Medical Legal Death Investigator (MLDI)**: This lecture covers what an MLDI is and their function relative to an investigation.
- **The Role of All Dentists in Forensic Dental Investigations**: This lecture covers the dentist's legal and ethical obligation during a forensic investigation, their role regarding record keeping, and their obligations to cooperate with the authorities when records are requested.
- **The Medical Examiner's Office**: This lecture explains the role of the Medical Examiner's Office within the community from monitoring illness to death investigations.
- **Imaging**: This lecture explains the role photography and radiology plays in victim identification and bitemark investigations and the pros and cons associated with forensic photography as it relates to dentistry.
- **Forensic dentistry software**: This session will expose dental students to the various types of software programs available to aid in victim identification of persons unknown and for managing mass disasters.

An optional rotation will provide an opportunity for students to visit the Northern Regional Medical Examiner's Office in Newark, New Jersey, to enable senior dental students to become familiar with the day-to-day workings of a medical examiner's office. Dental students must have attended the didactic forensic dentistry elective offered by the New Jersey Dental School. This is a unique opportunity for dental students to witness firsthand, as well as having a hands-on experience, in the field of forensic odontology. The rotation takes place over three consecutive days from 8:30 a.m. to 1:00 p.m and two students at a time participate in the rotation so as not to interfere with the day-to-day operations of the office.

The students shadow the medical staff of the office while they are performing their daily duties. On the third day of the rotation, each student is required to give a 15-minute presentation on dentistry/forensic odontology. The presentation is given to the Medical Examiner's office staff, medical students, and medical residents rotating through the Medical Examiner's Office. After the presentation, the students accompany dental school faculty associated with the Medical

Examiner's Office to the autopsy area. Students will participate in a hands-on experience of doing dental examinations and obtaining radiographs on a decedent.

Forensic Odontology, Curriculum, Education

F21 Aligned Education, Cooperative Learning, and International Collaboration in Forensic Dentistry

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After attending this presentation, attendees will gain a clearer understanding of the effects of international collaboration in the establishment and development of forensic dentistry in countries where this science has not received attention in the past.

This presentation will impact the forensic science community by raising awareness that, through aligned education, learning, international collaboration, and exchange, forensic dentistry can become an integral and vital part of police investigation work and disaster victim identification everywhere.

In the dental forensic community, it is a known fact that some countries are more advanced than others in utilizing forensic dentistry in crime investigation and/or disaster victim identification while others struggle to achieve a united vision and understanding among the different government agencies involved. Therefore, it becomes the moral and ethical responsibility and privilege of the more advanced forensic communities to engage in a collaborative effort to integrate this science into police investigation work in the less developed territories.

An example of this kind of international collaboration took place in Hungary where interest in the field of forensic dentistry first emerged at a time when little attention was given to this topic due to low criminal activity. Upon attending seminars and courses on the topic in the United States, including the symposium offered at the Center for Education and Research in Forensics in San Antonio, and through continued education, learning, and support from colleagues and mentors in the United States, this learning and experience was brought back to Hungary. The importance of bitemark analysis and victim identification was brought to light not only for the Hungarian Crime Scene Investigation Team but also for the general public through the mass media. Furthermore, a Disaster Victim Identification Forensic Expert Team was established which is now under the umbrella of the Hungarian Police Department. Additionally, the Hungarian Immigration Body is exploring possibilities to implement an age estimation program based on wisdom teeth development, a program originally developed and taught by the University of Texas Health Science Center at San Antonio.

This knowledge and awareness has not been limited to Hungary. The International Law Enforcement Academy (ILEA) in Budapest, which is run by the FBI, has asked for these topics to be incorporated into the training of their international police recruits and agents. Thus, the knowledge has been transmitted to Bulgaria, Kosovo, Romania, Macedonia, Montenegro, and other countries in Europe. The Semmelweis Medical University Department of Forensic Medicine in Budapest, which has integrated bitemark analysis, age estimation, and disaster victim identification into its curriculum, has contacted its partner agencies in Israel and, as a result, the National Israeli Police has so far arranged two workshops on bitemark analysis with follow-up programs for in the near future. Moreover, upon completion of their dental education at the Semmelweis University, newly trained dentists bring their knowledge and learning back to their home countries including Germany, Norway, Austria, Sweden, the United Kingdom, Greece, Cyprus, Iran, Israel, and other countries in the Middle East.

These incredible unforeseen accomplishments have been made possible because of the continued collaboration, exchange of knowledge and ideas, accompaniment and capacity building, and the support and encouragement received from mentors and colleagues working in the field in the United States. In facing common challenges and service to humanity, the concept of accompaniment and assistance in developing capacities are vital in chartering an individual path for progress. Great accomplishments in public service are achieved through unity of vision and action and mutual and ongoing collaboration and support.

Education, Learning, Collaboration

F22 Mass Disasters: Training Dentists in Switzerland

Michel M. Perrier, MS, Av de Rumine 7, Lausanne, SWITZERLAND*

The goal of this presentation is to present and discuss a model for continued training in odontology applied to the context of a mass disaster.

This presentation will impact the forensic science community by showing a model of continuing education in mass disaster management aimed at dentists.

In recent years, the variety and the quantity of mass disasters such as transportation accidents, terrorist attacks, bombings, earthquakes, and nuclear accidents have increased.

Accurate identifications of victims remain one of the critical issues in the management of any mass disaster. Most of the time, visual recognition is an insufficient and unscientific approach to obtain reliable results. Dactyloscopy, genetic analysis, and odontology are the best techniques that may lead to a positive identification. These methods need to be performed by specific experts along with the general autopsy.

The teeth are among the most resistant structures of the human body and thus represent a high potential of individualization in situations of highly decomposed, cremated, or skeletonized bodies.

In order to optimize the chances of obtaining reliable results, any postmortem, antemortem, and reconciliation procedure has to be carried out by an odontologist who has sufficient training in this discipline.

Any mass disaster involving a high number of victims may involve a sudden larger number of professionals to cope with the need of rapid and accurate identification.

Since 2006, the Medicolegal Institute of the University of Lausanne and the Disaster Victim Identification Unit of Switzerland has organized organizing courses designed as an introduction to the essentials of odontological identification. The participants learn the basics of identification: postmortem examination, analyses of antemortem records, and reconciliation. They are also exposed to the key features of odontological identifications based on fragmentation and commingling in the context of a mass disaster. They learn how to use the DVI digital identification system (PlassData).

Currently, the courses span two days. In 2006, 2007, 2009, and 2012, the program was advertised to approximately 400 dentists in the French-speaking part of Switzerland. It was also advertised in the monthly Swiss Journal of Odontostomatology. In each case, over 200 dentists responded but only 30 could enroll because of the limitations inherent to the organization of practical, hands-on exercises.

The speakers and other staff (odontologists, forensic scientists, and investigators) are appointed on the basis of their academic training, experience in the field of identification, and teaching experience. All of the specialists have actively participated in the management of several mass disasters.

The first day of the course comprises an introduction to legal medicine, to forensic odontology, and to the principles of odontological identification. This theoretical part is followed by a practical exercise of identification of human dentitions carried out by teams of two participants.

During the second day of the course, the participants are introduced to mass disaster management. After a theoretical introduction, practical exercises are carried out to simulate a mass disaster scenario in which mixed fragments of human dentitions have to be identified.

The activities and behavior of the participants as a whole group and within the teams are observed by the teaching staff that is present throughout the course.

Each participant receives a written evaluation form designed to assess some general features of the course, such as overall organization and the usefulness and quality of the provided information. In addition, each participant is asked to define his or her motivation in attending this type of training. Last but not least, each participant has to assess his or her readiness to become involved in the management of a real mass disaster event on short notice and for an indefinite period of time. Every questionnaire distributed to the participants is returned to the organizers for thorough analysis.

Any motivated participant is then invited to be involved in a continuing education system organized by the DVI of Switzerland to keep up.

The presentation consists of a critical assessment of the course's organization, follow-up, and the feedback that was received.

Odontology, Mass Disaster, Education

F23 "Ri.Sc." Database: The Italian Governmental Solution Regarding Missing Persons and Unidentified Cadavers

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After attending this presentation, attendees will have an understanding of the issues arising from Italian legislation on missing persons and unidentified bodies and how the collaboration of forensic odontologists can rapidly facilitate the identification process.

This presentation will impact the forensic science community by stressing the importance of a high degree of collaboration between police, the family of the missing person, and forensic odontologists.

Dental identification is one of the principle ways in which a cadaver can be positively identified. The results of a dental autopsy partially constitute the information which leads to the creation of a general biological profile through which initial compatibility testing with antemortem cadaver information may be executed.

In Italy, since April 1, 2010, in the Sistema Ricerca Scomparsi (Ri.Sc) missing persons search system database for missing persons and unidentified cadavers, the forensic odontologist has been able to play an active role with the medical examiner regarding the compilation of the Ri.Sc. form in order to collate pertinent dental data and oral radiographs of the cadaver with the goal of making a positive identification.

In order to test the functionality of the record system itself, the system was used on a sample of four unidentified cadavers (one well-preserved cadaver, one decomposed, one skeletonized, and one carbonized). The study revealed that the recording system allows for an immediate and simple method of data collection for the creation of a general biological profile while at the same time allowing for amplification of the odontological profile.

The forensic odontologist is of fundamental importance, not only in the analysis of the restorations *in situ* in the oral cavity but also for maxillary and dental radiography from which geographical origin, personal habits, and an estimated dental age can be derived.

Digital radiographs are easily archived and may be forwarded and used as an internal instrument of comparison for the Ri.Sc. system. The PM record "dental section" should be compiled by both a medical examiner and an odontologist.

This study underlines the importance of the dentist also being an expert in forensics so that all pertinent information may be retrieved and rendered useful for identification purposes and thus archived as a complete profile of the unidentified cadaver. This importance should also be underlined during the collection and coding of antemortem dental data during investigative procedures carried out by investigative police who do not have odontologists as part of their team of specialists who are routinely commissioned to deal with this type of data collection. With this in mind, a checklist to facilitate both the investigative police and the relatives of the missing person has been compiled regarding the collection of all dental data. The odontologist could also record information revealed during the postmortem exam on an appropriate international form, such as those suggested by Interpol, should the nationality of the cadaver be uncertain. Interpol forms are aimed at the identification of mass disaster victims, but in light of the lack of an international standard for antemortem and postmortem odontological data and the need to overcome language barriers and diversities in foreign legal systems, they are also an excellent way of fostering the mutual exchange of information which may lead towards the positive identification of unidentified human remains.

Missing Persons, Human Identification, Forensic Odontology

F24 What Are We Accomplishing With Dental Information? Obstacles Facing Forensic Odontologists and Working Solutions

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After attending this presentation, attendees will be able to understand the importance of a multidisciplinary approach to entering dental information into national databases, such as the National Crime Information Center (NCIC) and the National Missing and Unidentified Persons System (NamUs). Additionally, attendees will be able to understand the obstacles encountered and the solutions needed in the process of ensuring accurate dental coding.

This presentation will impact the forensic science community by emphasizing the importance of working with law enforcement, medical examiners/coroners, and forensic odontologists. In this way, forensic odontologists will be able to acquire, evaluate, interpret, and code missing and unidentified person's cases. Furthermore, through NamUs, families of missing persons are now aware that vital information of their loved ones is available in national databases, providing hope for future identification. It is not uncommon for potential matches to be made through the information provided by family members who routinely search the Internet. For this reason, it is vital that a protocol be provided that will ensure that the dental information is accurately entered into the appropriate databases in hopes of securing a positive identification.

The initial phase of this initiative concentrated on the NCIC dental coding entries and their inaccuracies. In identifying this problem, protocols were phased in to correct coding and thus ensure dental accuracy in NCIC. The initiative obtained original dental records and radiographs, provided coding review by experienced forensic odontologists, and digitized dental radiographs.

The second phase of this initiative now encompasses over 200 cases entered in both NCIC and NamUs. Observations and experiences since the initial phase have called for modifications and streamlining of protocols due to the prevalence of miscoded dental data. The accuracy of New Jersey's dental information has greatly improved in NCIC and NamUs since the inception of this initiative which can better assist in additional positive identifications; however, challenges remain. There have been many obstacles that have been encountered in continuing this initiative. These include the lack of reviews by more than one experienced forensic odontologist when coding missing and unidentified persons, the recognition that family dentists are unfamiliar with the different database codes, thus providing inaccurate dental information, and the awareness that law enforcement working missing person cases rely on the information provided by the family dentist. These and other obstacles will be discussed. Ensuring accuracy is still of paramount importance to this initiative in order to provide an avenue for positive identification.

By identifying these obstacles during this second phase, there is currently a concentration on creating training models in dental coding to forensic odontologists, education models for the law enforcement community, and on networking with families and family dentists. Solutions to these challenges such as access to additional training and more experience and familiarity with the coding systems for missing and

unidentified persons will also be discussed. This presentation will discuss the information learned throughout this second phase. It is anticipated that the obstacles and possible solutions presented in this presentation would help to assist other jurisdictions in the productive management of dental information into our national databases. This can only improve the identification of unidentified persons in this country and around the world. An overall update on the current status of this initiative will be presented.

Dental Information, NCIC, NamUs

F25 Contrast Enhancement of Bitemark Images Using the Greyscale Mixer in ACR in Photoshop®

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After attending this presentation, attendees will have an understanding of the method employed in this experimental image enhancement technique and be aware of the limitations in use as evidence.

This presentation will impact the forensic science community by helping investigators research the benefits and limitations of image enhancement techniques in analysis of bitemarks and patterned bruises.

Forensic odontology can best be defined as the overlap that represents the dental and legal professions. Bitemark analysis is one aspect in which a forensic dentist can assist the courts. Teeth are often used as weapons, and the resultant bitemark can aid forensic odontologists to exclude or otherwise appraise a suspect's dentition. Claims that the size, shape, and pattern of the biting edges of the upper and lower anterior teeth are specific to an individual have recently been challenged, leaving the question of uniqueness of the dentition unresolved.

Analysis of bitemarks for the purpose of identifying a perpetrator is further compounded by distortion introduced into the bitemark and its record from the moment of infliction. Researchers have recently revisited the concept that skin is a poor impression material and the resultant difficulties that this imposes on bitemark analysis. Movement during infliction and the lapse of time also introduce distortion, which complicates further the interpretation of a diffused patterned bruise.

In recent years, the advent of digital photography has, among other things, made possible the application of digital enhancement to an image, thereby increasing the possibility of achieving better edge definition for analysis. A number of studies have demonstrated such use of computer software to enhance the edge definition of patterns within images in various forensic applications. Some examples include color separation, which uses algorithms within Adobe® Photoshop® for removal of distracting background patterns from fingerprint and handwriting evidence, and Red, Green, Blue (RGB) channel extraction, which removes one or more of the three RGB channels to decrease background interference in a variety of crime scene images. A similar method is used in footmark analysis where details are extracted and the background information is reduced by viewing the image separated into RGB and Cyan, Magenta, Yellow, Black key (CMYK) color channels. The layers containing information or distractions can be identified and, following a mixture of blending and removing color layers, the subject can be made clearer.

In an attempt to apply more subtle and precise enhancement methods to the analysis of bitemark images, a novel contrast enhancement technique based on the adjustment of the brightness pixel values, similar to that attained by the level or curves function in Photoshop® was developed. This studies goal was to acquire initial data on the end user's preference for either RGB color, greyscale, or the grayscale-enhanced images in order to validate the benefit of this contrast-enhancement technique. With the understanding that there are other inherent problems affecting bitemark analysis, which will not be overcome by applying edge-definition enhancement. The suggested technique goal is to widen the range of tools available to the expert when looking to undertake bitemark analysis.

Enhanced images may improve bitemark edge definition, assisting forensic analysis. Current contrast enhancement involves color extraction, viewing layered images by channel. A novel technique, producing a single enhanced image using the greyscale mix panel within Adobe® Camera Raw®, has been developed and assessed here, allowing adjustments of multiple color channels simultaneously. Stage 1 measured RGB values in 72 versions of a color chart image; eight sliders in Photoshop® were adjusted at 25% intervals, all corresponding colors affected. Stage 2 used a bitemark image, and found only red, orange, and yellow sliders had discernible effects. Stage 3 assessed modality preference between color, greyscale, and enhanced images; on average, the 22 survey participants chose the enhanced image as better defined for nine out of ten bitemarks. The study has shown potential benefits for this new technique; however, further research is needed before use in the analysis of bitemarks.

Bitemark Analysis, Contrast Enhancement, Adobe® Photoshop®

F26 Lip Prints: The Argument for Research in Cheiloscopy

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The goal of this presentation is to report and describe the existing research on cheiloscopy. Attendees will discern that there is sufficient evidence from the literature to determine the need for further efforts to include lip prints as a means to identification.

This presentation will impact the forensic science community by demonstrating the potential cheiloscopy has on identification as a sole or supportive paradigm. The forensic science community will be asked to accept the uniqueness and forensic value of lip prints as a legitimate identification method. The argument will be made for the need of further study in order to use cheiloscopy as a means of identification and possible adjunct in bitemark analysis.

A very different, less known, and unconventional form of identification is presented. Cheiloscopy is the study and examination of lip prints. The human lips have a pattern as do fingers, palms, and feet. They have been referred to as grooves, wrinkles, lines, and creases with extremely fine details. It is a form of impression evidence similar to ear prints and elbow prints. Of late, these have been the topic of varied research and are also occasionally mentioned in literature by a detective, crime scene investigator, or an examiner in another field of evidence comparison. Subject comparisons have been recorded in the thousands. They have been done at universities using students and in specific communities throughout the world. Research studies on lip prints have been contributed mainly by forensic dentists and anthropologists in countries throughout the world. This includes, but is not limited to, Brazil, Spain, Germany, Russia, Japan, Canada, India, Saudi Arabia, France, Italy, Great Britain, Iran, Czechoslovakia, and Poland.

One method of comparison appears often. It was designed by Tsuchihashi in 1970. The lips are divided in segments and the grooves are typed and compared. Examiners determine if the pattern is Type I—vertical, Type II—Branched, Type III—Reticular, or Type IV—Undetermined. There has been much success in identification via the recorded prints on cellophane tape and white paper. This method has been reported exclusively in the recent research. Researchers have also been studying the patterns more associated with gender and raise the possibility of determining gender from lip prints.

The search for existing studies was made through Pub Med, and Scopus using the search words "lip prints" and "cheiloscopy." There were 35 retrieved. Thirty-three were in English, one in German, and one in Russian. These articles range from the years 1970 to 2011. In order to evaluate the latest published reports, the bulk of the articles retrieved (24) were published from 2005 to 2011. Some of the publications included: *Journal of Forensic Odonto-Stomatology, Forensic Science International*, *Indian Journal of Public Health Research & Development*,

Journal of Forensic Dental Sciences, and *Journal of Forensic Identification*.

The key to the argument is in latent lip prints, those not made by any cosmetic material and unseen by the naked eye. These are not usually "dusted" at crime scenes. Nor have they been considered when a bitemark is present. Research would be able to aid in finding out whether or not the latent lip print can be lifted around a bitemark on human skin. A forensic pathologist, Esperanza Navarro, and others in Spain researched this on cadavers using invisible lipstick (commercially obtained cosmetic) but the need is present to test on human subjects. Studies should include lip prints using lipstick, and the latent prints made by invisible lipstick and no cosmetics at all. If this can be done, it would be an enormous adjunct to bitemark analysis. The potential to match lip prints to a suspected biter would further validate bitemark analysis. A proposal will be made to a university IRB committee in the interim.

Cheiloscopy, Lip Prints, Bitemarks

F27 Study of the Palatal Rugae Patterns Among the Indians and Chinese at Manipal, India

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After attending this presentation, attendees will: (1) gain knowledge about the existing problem of identification in mass disasters and the role of a forensic odontology in relation to this; (2) be briefed on the various comparative data and techniques that are useful in establishing the identity of an individual with specific stress on the upcoming field of palatoscopy or rugoscopy; and, (3) gain information on the various palatal rugae patterns in an individual and application of this knowledge in a given population to aid in establishing identity.

This presentation will impact the forensic science community by shedding new light on the less explored field of rugoscopy. It provides sufficient information of the biological diversity present in this part of the Indian subcontinent. It may prove useful in comparison to other studies of a similar nature and can be applied to various other populations. This research will arouse an interest in others to conduct a similar study in their respective countries which may go a long way in aiding the establishment of the identity of an individual. In other words, we, the medical community, will be better prepared in facing mass disasters to help mankind.

In this world of natural and man-made disasters, identification of an individual becomes an important task for any Disaster Victim Identification team. In almost every disaster, there is an urgent and pressing need to identify the victims on behalf of the next of kin. Comparative data/techniques play a vital role to aid in identification.

Palatoscopy or palatal rugoscopy is the name given to the study of palatal rugae in order to establish a person's identity.[1] Despite the ongoing problem of describing palatal rugae pattern, qualitatively and quantitatively, their uniqueness to individuals has been recognized as providing a potentially reliable source of identification.[2-4] Rugae patterns have been studied for various purposes mainly in the fields of anthropology, comparative anatomy, genetics, forensic odontology, prosthodontics, and orthodontics.[5] The present study is an attempt to compare the palatal rugae patterns among the Indians and Chinese students at Manipal from a small part of peninsular India.

Maxillary dental casts of 63 Indian (32 males, 31 females) and 61 Chinese (31 males, 30 females) students of the age group 17 – 23 years were assessed for the length, shape, and unification of rugae based on the classification by Thomas and Kotze (1983).[6] Association between rugae forms, ethnicity, and gender were tested using 2-way ANOVA (Univariate Analysis of Variance) and contingency tables.

Statistical analysis showed the total number of rugae on the right side of the palate was greater among the Indians than the Chinese. Females had a larger number of curved and straight rugae whereas males had more wavy rugae in both races. The incidence of rearward-directed rugae was greater among the Chinese females and Indian males than their respective counterparts. There was no significant difference in the converging and diverging type of rugae among the Indian and Chinese populations of both sexes.

The statistical analysis of the data revealed significant differences among the Indians and Chinese. Rugae patterns are complex and are so open to individual interpretation that a very careful observation is essential. These patterns are highly individualistic and could form a useful tool to aid in identification, especially in cases of mass disasters.

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Mass Disaster, Identification, Palatal Rugae

F28 Anterior Bite Force and Association of Bitemarks with Dental Models

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After attending this presentation, attendees will be able to: (1) measure maximum voluntary anterior bite forces, generated at baseline and aroused emotional states; (2) assess force consistency from day to day; and, (3) review the effect of an emotional state on bite force to assess whether dentists with forensic experience can match photographs of wax bite impressions to corresponding dental models.

This presentation will impact the forensic science community by providing foundational data to direct future research that evaluates the validity of human bitemark analysis in criminal investigations.

Teeth are used as weapons for offensive (perpetrator) and defensive (victim) purposes, and bitemark injuries are often evident on victims of sexual assault, child abuse, and homicide.[1] Assailants often use their front teeth to inflict injuries. Although bitemarks are analyzed in criminal investigations, the science supporting bitemarks as evidence is weak.[2] In addition, bitemark analysis is confronted by numerous controversies and challenges.[3-5] How emotion influences anterior bite force is unknown, and whether experts can correctly associate bitemarks and dental models has not been substantiated.[6] An improved understanding of the factors that affect how and why human bitemark injuries are made will enhance analysis of bitemarks as evidence during investigation and prosecution of such crimes.

Phase 1 of this pilot study measures maximum voluntary anterior bite forces, generated at baseline and aroused emotional states, to assess: (1) force consistency from day to day; and, (2) the effect of an emotional state on bite force. Phase 2 assesses whether dentists with forensic experience can match photographs of wax bite impressions to corresponding dental models.

Phase 1: Thirty participants will have stone dental models fabricated from full arch dental impressions. Participants will make four maximum anterior force bites on a wax-covered gnathodynamometer on two separate days (two bites each day). They will be asked to rate their baseline stress level on a 100mm visual analogue scale (VAS) with 0 = no stress and 100 = the worst stress imaginable. Stress will be

assessed before each bite. Before the fourth bite, each participant will be asked to recall an event that aroused anger (and re-estimate their stress level). Photographs will be taken of the wax impressions made by the second and fourth bites. Phase 2: Five dentists with forensic experience will attempt to match photographs of the wax bite impressions to corresponding dental models.

Phase 1: Mean bite force will be calculated for the four bites and compared using Repeated Measures ANOVA and Tukey HSD post hoc tests (α=0.05) to determine (1) if baseline bite forces (bites #1, #2, and #3) differ from one another, and (2) from the anger induced bite force (bite #4).

Phase 2: The number of correct matches will be recorded and percent agreement calculated for each examiner. Kappa statistics will assess the level of agreement.

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Bitemark, Bite Force, Anger Arousal

F29 Transfer of Dental Patterns to Human Skin

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After attending this presentation, attendees will: (1) understand how tooth characteristics may not be reliably transferred and recorded in the bitten subject; (2) understand that alterations in height and displacement of particular teeth may affect the position of adjacent teeth in the bitten subject; and, (3) be introduced to additional distortion in a bitemark.

This presentation will impact the forensic science community by providing a better understanding of how individual tooth characteristics are recorded in bitten tissue.

Previous studies on human cadaver models have reported significant levels of distortion of bitemarks in skin, and matches among the anterior dentition in open population studies have been found. The goal of this study is to establish a threshold as to what degree of difference in shape of one dentition will be distinguishable from another as reflected in a bitemark in human cadaver skin.

HSIRB exemption was granted for this project. Eight sets of dental casts were produced and divided into two groups. One group varied the affects of height changes in teeth while the other varied tooth displacement affects. In the first group, the lateral incisors were systematically shortened in 1mm increments up to 3mm. In the other group, the lateral incisor and canine were facially/lingually displaced in 1mm increments up to 5mm. Each of the models was scanned on a flatbed scanner for comparison with the bitemarks they produced.

A series of ten repeated bites, distributed over arms and legs of unembalmed cadavers, were inflicted with each model, and digitally photographed with an ABFO #2 scale in place.

Comparison analysis using landmark-based geometric morphometrics indicated that alterations of a limited number of teeth not only introduced additional distortion in the bitemark, but also affected the apparent position of adjacent teeth in the bitemarks. Displacing the lateral incisor and canine resulted in a relative shift in position of the central incisors and unaltered canines, while the shortening of the lateral incisors resulted in a shift in relative position of the central incisors. Moreover, the features of displacement were more pronounced in the inflicted bitemarks than in the dentition used to make the bites, thus the bitemarks tended to exaggerate the differences. Also, the observed distortion was more significant in the mandibular than maxillary arch, suggesting that the mandible expresses higher variation than the maxilla.

Thus, due to shift in relative tooth position and resulting distortion, nearly every bitemark consequently looked unique, and no matches within measurement resolution were found between the bitemarks and the dentition used to inflict them. It was found, however, that a displacement of 5mm between teeth enabled distinction between the dentitions. No such threshold of distinction could be established for the height variation of teeth under the given experimental conditions. In other words, it was not possible to establish a reliable threshold of how different dentitions have to be in order to be distinguishable from one another.

The study was conducted under a controlled situation on cadaver skin with limited variables. In a real biting situation, more variables are involved, such as dynamics of the bite or movement of the victim, which would possibly lead to more distortion of a bitemark than under the controlled conditions of this study. The results of this study indicate that bitemark evidence should be used with caution.

Bitemarks, Bitemark Research, Distortion

F30 Violating the "Biologic Width" — Is It Worth It?

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After attending this presentation, attendees will comprehend the importance of the natural biologic width of gingival attachment that exists in human dentition, and why it is necessary to determine its actual dimensions prior to crown lengthening procedures. Violation of the biologic width leads to post-operative gingival hyperplasia, bleeding, and severe pain.

This presentation will impact the forensic science community by educating odontologists engaged in civil dental malpractice case review and establishing the criteria for understanding the importance of maintaining the biological width, accurate documentation, and record keeping during their own general and prosthetic work, and assist in the civil litigation cases they may review in the course of forensic investigation of dental malpractice. A case presentation will photographically depict and verbally describe the events and procedures leading to a failed prosthetic reconstructive result, investigation of the post-operative consequences with a conclusion of gross negligence and perjury by the defendant, and resolution of the treatment performed by the defendant.

Traditional crown lengthening is a frequent procedure used to reduce excessive gingival display (gummy smile).[1,2] Treatment involves close coordination between the treating restorative dentist and the periodontist in order to protect the biologic width of the connective tissue attachment. This is pre-determined by sulcus depth probing wherein the biologic width is sounded and a periodontal flap is used to expose the root surfaces and alveolar bone.[2,3] Bone is then removed and the gingiva repositioned according to the appropriate width of the attachment.[4,5]

A 39-year-old female patient consulted a well-known and highly profiled cosmetic dentist from Orange County, California, for treatment of her gummy smile and repair of a discolored front tooth which had an unsightly fracture sustained as a teenager. The dentist then proceeded to complete 3mm gingival recontouring by laser surgery and preparation of 22 teeth in two hours and forty minutes, resulting in severe pain in several of the patient's teeth. Following a series of unsuccessful subsequent appointments, the patient and her husband determined that they should seek second opinions from several specialists in the area.

It was concluded that not only were several teeth over-prepped, which required eight root canals to alleviate pain, all crowns needed to be removed and replaced after correct crown lengthening, along with correction of a malocclusion created by the original restorations.

The litigation by binding arbitration occurred over seven days, wherein experts from both plaintiff and defendant testified to their opinions. During testimony by the defendant, it was found that records were falsified and that pre-treatment and post-treatment photos and models had been purposely destroyed by the defendant.[6] Subsequent damages award, current and future economic loss, and court costs were given to the plaintiff in excess of $700,000.

This case study is an example of what may occur after neglecting the importance of the biologic width in prosthetic and reconstructive cosmetic dentistry, and the need for maintaining accurate records.

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Civil Litigation, Dental Malpractice, Forensic Odontology

F31 Dental Death — Unfortunate Fatal Results From Errors of Omission and/or Commission

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After attending this presentation, attendees will learn the serious and fatal results from both medical and dental errors in judgment. They should learn the importance of making a proper diagnosis, as well as taking a complete history and treatment plan for what may seem a minor dental condition.

This presentation will impact the forensic science community by discussing the seriousness of dental infection, and how the lack of proper and timely treatment can lead to the death of a patient. Likewise, the omission of following the American Heart Association (AHA) and American Dental Association (ADA) standards for pre-medication may and can result in the death of a patient. By recognizing the serious consequences of not following proper preventive procedures, the practitioner should be cautious and mindful to avoid the errors of omission and/or commission that can result in fatal consequences.

This study covers over 35 years experience in reviewing over a dozen cases of death from dental treatment or lack thereof, all the cases having been ruled natural as to manner of death. The causes range from SBE (sub acute bacterial endocarditis) secondary to dental treatment, subdural empyema from dental infection or CVA (cerebral vascular accident) from discontinuation of anti-coagulant medications. Dental infection, whether from an abscessed tooth, periodontal disease, or defective root canal treatment can result in systemic infection and death of a patient. The improper protocol for IV sedation has had the unfortunate result of death of the patient. Does this breach of the standard of care rise to the level of manslaughter? The dental professional has a duty to his patient to know his/her medical history and always consult with the primary physician when there is a question or need to alter medications. The injection of local anesthesia containing epinephrine should be used with extreme caution when treating medically compromised patients such as ones with pulmonary edema.

Pre-medication requirements have changed over the years from one gram twice a day for one day before, day of, and day after an invasive procedure, to today's single dose of two grams one hour before procedure. For patients at risk, not following the proper protocol may have fatal results. Something as simple as a restoration at the crest of the gingival—a Class V—can cause bleeding and thus require the necessary pre-medication protocol. In cases of patients with prior rheumatic fever, damaged heart valves, or those with any type of valve replacements, any invasive dental procedure such as extractions, implants, cleanings, certain fillings in sub gingival cases, and crown preparations may seed the *Streptococcus Viridans* bacteria that is present in the oral cavity and produce SBE (subacute Bacterial Endocarditis). The dentist is responsible for taking precautions with this infection and must follow the AHA's and ADA's guidelines.

It seems only logical that a medical history be taken before treatment is instituted and reviewed for any potential problems of the proposed treatment. Why would a dentist perform an extraction in the presence of cellulitis, a history of dizzy spells, weight loss, constant severe unilateral headaches, and not take a temperature, review prior medical history, and make a timely referral? A simple extraction of a wisdom tooth on a 23-year-old male did not cause his death. But the undiagnosed systemic infection with subdural empyema present did. All dental practitioners need to be more aware of the seriousness of dental infections, reaction to anesthetics, importance of medical history, and strict adherence to pre-medications in order to avoid these tragic consequences.

Empyema, Manslaughter, Cellulitis

F32 Passing *Voir Dire* and Avoiding Cross-Examination: A Follow-Up Case Report

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The goal of this presentation is to show how the forensic odontologist must be able to defend in court whatever forensic evidence was promulgated by established methodology.

This presentation will impact the forensic science community by encouraging forensic odontologists to be aware of the techniques involved with being an expert witness.

This case was used as an example of proper coding techniques presented at the American Academy of Forensic Sciences' Annual Scientific Meeting in February 2009, by Dashkow, Fontana, and Dondero. This is a follow-up presentation to highlight the litigation techniques used especially in preventing a possibly devastating cross-examination experience.

In July of 2008, skeletonized remains were discovered in a wooded area adjacent to the Southern State Parkway, in Nassau County, New York. A preliminary estimate based on the extent of decomposition and local climate conditions fixed the time of death at six to eight months. Initial examination by a forensic anthropologist was that the remains were of a Caucasian female approximately twenty-eight to thirty-two years of age. A forensic pathology examination determined a cause of death to be by "blunt force trauma" to the skull resulting in numerous fractures of the cranial structures. Most significant was a fracture extending from the left frontal-parietal suture across the frontal bone through the right orbital ridge. The New York State Police could not find any missing person from this period to match this individual.

Initial forensic dental examination revealed an intact maxilla with all sixteen teeth present. Several amalgam restorations were noted. Seven digital periapical radiographs were taken and a clinical dental chart was produced. Teeth numbers seven and nine were subsequently removed for DNA analysis.

The intact yet disarticulated mandible was then examined. All teeth were present except for numbers twenty-three, twenty-four, and twenty-six, which appeared to have been lost through postmortem evulsion. Several amalgam restorations were also noted to be present. What was noted immediately was the presence and full eruption of both third molars as well as an impacted supernumerary "fourth" molar located distal to both third molars. Seven digital periapical radiographs were taken and a clinical dental chart was produced.

The State Police investigator was notified of the uniqueness of the decedent's dentition. The investigator contacted the New York State

Dental Society which agreed to send an email to its members asking if anyone had possibly examined a patient with such a dental anomaly. Dr. Frank Pappas received the email and forwarded it to a New Jersey colleague, Dr. Sheila Dashkow. Dr. Dashkow recalled entering a missing person's dental information with similar characteristics into the database and contacted the New York State Police investigator who forwarded this information to Dr. Henry Dondero. Dr. Dashkow emailed a copy of the missing person's panoramic radiograph and a tentative identification was made. Several days later, the original radiograph was brought to the Nassau County Medical Examiner's Office by the State Police where the identification was confirmed and an affidavit of Dental Identification was completed.

In April 2010, Dr. Dondero was notified by the Bergen County Prosecutor's Office was notifed that testimony would need to be required in this case. This case was mainly circumstantial in nature and the forensic evidence overwhelmingly corroborated the prosecution's assertions. The prosecutor was concerned that the defense would be highly critical of all the forensics. In preparation for trial, the procedures for dental identification were reviewed as well as Dr. Dondero's *curriculum vitae* which was anticipated to be scrutinized in *voir dire*. During opening statements, the defense postulated that the forensics were faulty because of the time difference between the approximate date of the crime, October 2007, and discovery of the remains, July 2008. Even the circumstances surrounding the identification would be questioned.

At trial, the prosecution spent considerable time reviewing many details contained in the *curriculum vitae* before making a motion to have the testimony accepted as an expert witness, which he was without objection. In describing the identification process every procedure was explained in detail and the prosecution asked very pointed and probing questions. Antemortem and postmortem radiographs of every restoration were compared and shown to the jury. The pedigree of the original radiographs was documented as well as the unique search for the missing person record. No cross-examination was forthcoming and all dental evidence was accepted.

***Voir Dire*, Cross-Examination, Litigation**

F33 Developing Age Estimation Standards for a Western Australian Population Using the Coronal Pulp Cavity Index

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After attending this presentation, attendees will understand the principles and application of the coronal pulp cavity index method for age estimation. This is a non-invasive and simple age estimation method based on secondary dentin deposition. It has significant forensic applications because this method can be applied in living individuals who do not have the appropriate proof of age documentation.

This presentation will impact the forensic science community by describing the process of formulating age estimation standards specifically developed for a Western Australian population. As a result of increasing global mobility, contemporary age estimation standards are required and this study contributes to the body of information in this regard. This method facilitates in chronological age estimation in living individuals based on an uncomplicated and non-destructive method.

Age estimation is an integral aspect of forensic odontology and a crucial element toward establishing the positive identification of living individuals and skeletal remains. In modern multicultural societies where legal and illegal immigration is rising, an increasing demand exists for age estimation in living persons who have no documentation for proof of identity. Age estimation methods, such as aspartic acid racemisation and cementum apposition, require sophisticated laboratory techniques and are time consuming. Furthermore, these methods analyze extracted teeth and, hence, are unsuitable for application in living individuals and/or situations where extractions are not possible for religious and cultural reasons. Simple, non-invasive, and accurate methods are therefore required for age estimation in adults to develop population-specific standards.

Previous research has verified the association between age and secondary dentin deposition. The subsequent reduction of the coronal pulp cavity and its correlation with chronological age is assessed in a Western Australian population following the method of Drusini et al.[1] A total of 450 digital orthopantomograms (220 female and 230 male) from a Western Australian population were analyzed. The age range was 9 to 60 years with a mean age of 28.33 years for females and 27.71 years for males. The crown height (CH) and coronal pulp cavity height (CPCH) was measured in mandibular premolars and molars (excluding third molars) using the in-built visualization software. The tooth coronal index (TCI) was calculated using the formula TCI=CPCH x 100/CH. Linear regressions were performed by regressing the TCI against chronological age. The standard error of estimate (SEE) for the pooled sample was lowest at ±9.64 years for the mandibular left first and second molars. This was followed by the mandibular left second molar and first molars at ±10.74 and ±10.78 years respectively. In females and males, the SEE was lowest for mandibular left molars at ±9.54 years and ±9.74 years respectively. The SEE values obtained by Drusini et al. for their pooled sample ranged between ±5.88 years for the right side molars, to ±6.66 years for the left side premolars.[1] Although the SEE values in this study are considerably higher than those obtained by Drusini et al., these standards will be useful in classifying a Western Australian individual within adult age groups (i.e., young, middle, and older adult).[1]

The level of accuracy achieved using the coronal pulp cavity index is higher as compared to methods using skeletal markers, such as the sternal extremity of the fourth rib (SD=14.93 years in an American population) and the pubic symphysis (SD= 12.4 and 12.2 years for females and males respectively in Phase VI).[2,3] This study represents the first ever investigation of this method in a Western Australian population and the results indicate the method is suitable for forensic application.

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Age Estimation, Secondary Dentin, Western Australia

F34 A Comparison Between Restorative and Flowable Resins at Varying Temperatures Using X-Ray Fluorescence

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The goals of this presentation are to describe: (1) the uniqueness of inorganic properties of various resin materials; (2) the change in properties of resins when exposed to high temperatures; and, (3) the significance of these findings as they relate to victim identification.

This presentation will impact the forensic science community by increasing understanding of how documentation in the dental chart of the brand names of the restorative materials used is critical and may further strengthen the ability to provide positive identifications of remains.

With cosmetic dentistry becoming more in demand, the use of amalgam has steadily declined over the past decade. Although more esthetically pleasing, resin composite restorations pose a unique challenge to the forensic dentist when it comes to identifying remains, as they can be difficult to see clinically and on radiographs. If the remains have been incinerated, visual identification of restorations becomes

even more challenging. Resin composites are comprised of two primary components: organic monomers and inorganic fillers. It has been shown that the inorganic composition is, for the most part, unique to the brand.[1] The use of an SEM with Energy Dispersive X-ray Spectroscopy (SEM/EDS) has proven useful in identifying the elemental composition of specific resin restorative materials.[2] However, the SEM/EDS is a large machine, and it is not practical for a field or morgue setting. A portable X-ray Fluorescence spectrometer (XRF) can be used to overcome this challenge, and still correctly identify resin composition.[3] This table clinic will use XRF to determine compositional differences among three brands of conventional and flowable resin composite materials at various temperatures.

Three brands of conventional and flowable resin composites were chosen: Filtek Supreme (3M ESPE), Esthet-X (Dentsply), and TPH3 (Dentsply). Twelve disks of resin material (two from each type/brand), 1cm in diameter, were prepared and cured according to manufacturer's instructions. Six individual disks (one from each type/brand) were then placed in a ceramic crucible and placed in a burnout oven and heated from room temperature to 300°C, 600°C, and 900°C, with a 30 minute hold at each temperature. The portable XRF was scanned over the disks both at room temperature and after being heated at each of the three target temperatures. The X-ray spectra were compared to the control disks and to the manufacturer's stated elemental compositions.

Dental identification can be challenging in the best of circumstances. When remains are incinerated, the ability to obtain fingerprints and DNA is either gone or greatly diminished. This places the burden of scientific proof on the forensic dentist. The antemortem dental record, including both written text and radiographs, is an absolutely critical piece of the identification puzzle. Because the inorganic components of resin composites are unique to the type and brand of material, accurate documentation in the dental chart of the brand names of the restorative materials used is critical, and may further strengthen the ability to provide positive identifications of remains.

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X-Ray Fluorescence, Resins, Dental ID

F35 The Use of the Incise Dental Scanner to Compare Bitemarks in Cheese With Models of the Suspects' Dentition

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After attending this presentation, attendees will become better aware of how digital imaging techniques can be used in the quantification and analysis of bitemarks.

This presentation will impact the forensic science community by providing insight into how the application of digital imaging technology may help in the more objective assessment of deformations caused by weapons or tools in the investigation of crimes.

This study presents a technique developed for 3D imaging and quantitative comparison of the human dentition and bitemarks created in a perishable substrate, cheese. Six adult volunteers from the biometric lab, Queen Mary, University of London, had study casts made of their dentition and also bit into 40mmx20mmx20mm blocks of cheese. Impressions of the bites were made in putty fixture (Extrude, type 0, Germany). The dental models and the corresponding bite impressions were digitized by a contact coordinate measuring machine, (Incise Dental Scanner). The digitization strategy was set as follows: 1mm-diameter probe tip of 30mm length, scanning at Y direction, 0.1mm scanning interval, and scanning speed of 500 points per minute. The dental and bite models from which the incise Dental Scanner can capture coordinate data, from these data points, produces a model of the surface, thus providing a means of obtaining objective and accurate measurements and comparison of morphology.[1] Sets of dental models and corresponding bite impressions were numbered. This technique allows pattern association comparison to be made between the 3D images of the dental models and a bitemark.

Data analysis was based on the use of a 3D image analysis software package Cloud (UCL).[2] It is a versatile tool used to reconstruct 3D coordinates (X, Y, Z) into a color digital image for visualization and to analyze a free form surface. Cloud software has several tools for digital model analysis and measurement. It provides functions of superposition, calculates the geometric shapes, and analyzes the geometric relationship of surfaces. Superposition as a method of comparison is the procedure of bringing geometric objects into appropriate alignment but is dependent on the quality of the 3D digitized images of these objects.

The biting edges are usually the only clear feature in a bitemark and, therefore, were selected as the reference frame for the comparison. Based on the mathematical least square within the common area in the registration procedure using the Iterative Closest Point (ICP) algorithm within the software, the two surfaces were superposed; at this superposed position the difference along the surface at each single digitized point then was calculated and displaced on the computer screen corresponding to the cursor position on the image.[3] Different colors present depth differences between the surfaces: zero differences represented by sea blue color; +10µm to +20µm differences presented by orange, red, and purple; black colors represent -10µm to -20µm differences. Furthermore, the Cloud software provides the absolute numerical differences between the two superposed images; these differences demonstrate the distribution of the differences in relation to every single registered point.

A pattern-associated comparison using the superposition method between the 3D digitized images of the dental models and their corresponding cheese impressions was performed demonstrating a high degree of similarity of the incisal edges outline confirmed to be produced by the corresponding model. The descriptive statistics revealed that the average differences between the dental models and their corresponding bite impressions range between 0 and 3µm and mode between ±12µm. The standard deviation and the square root of the mean were exactly the same which recorded a range between 9µm and 11µm. These findings agree with the distribution of the absolute differences between the images which was extracted from the subtracted image and plotted using bar graph; the data was in bimodal shape with the peaks falls around ±10µm.

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Bitemark Analysis, Incise Scanner, Superposition

F36 What Should Counsel Do in a Bitemark Case? Competency of Counsel Issues in the Post-Conviction Context

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After attending this presentation, attendees will understand the prevalence of wrongful convictions in cases that contained bitemark evidence and guidelines for defense counsel who engage in seeking out and investigating these cases post-conviction.

The presentation will impact the forensic science community by serving to identify the very serious and continued implications of prior flawed bitemark evidence and providing those seeking to rectify wrongful convictions guidelines to do so in order to end the continued suffering of those convicted on the basis of such evidence.

The scientific validity of bitemark comparisons has been challenged for many years. In 1985, two researchers wrote: "crime-related bitemarks are grossly distorted, inaccurate, and therefore unreliable as a method of identification." Similar conclusions were reached in a study of wrongful convictions. Finally, those criticisms were echoed in the recently published study of the National Research Council entitled "Strengthening Forensic Science in the United States: A Path Forward" (NAS Report). Of the nation's 941 known exonerations, 225 involved false or misleading forensic evidence, including bitemark evidence. There are numerous examples of people who were convicted on the basis of bitemark analysis and later exonerated by subsequent DNA evidence. The DNA exonerations of Ray Krone, Bennie Starks, Kennedy Brewer, Roy Brown, Willie Jackson, James O'Donnell, and Calvin Washington provide clear evidence that bitemark evidence continues to be a cause of wrongful conviction across the nation.[1-4]

It is important to note that, despite the documented problems with bitemark "matches," bitemark exclusions are still considered reliable and can be extraordinarily helpful in the post-conviction context. For example, the NAS Report states: "Despite the inherent weaknesses involved in bitemark comparison, it is reasonable to assume that the process can sometimes reliably exclude suspects." Similarly, Wilkinson's and Geroughty's article points out: "It is easier to conclude that a person's dentition and a bitemark do not match than it is to find a match. This is due to the fact that any unexplained inconsistency between the bitemark and the dentition means that the suspect could not have made the bitemark." One case currently pending in front of the California Supreme Court is that of William Richards. In 1997, Richards was convicted of killing his wife, Pamela. In his third jury trial (the other two resulted in hung juries), the prosecution, for the first time, introduced evidence which suggested that Richards was responsible for a bitemark found on Pamela's hand and that only 2% of the population had a dentition (like Richards') which could have made that bitemark. In a later post-conviction hearing, Richards proved that the bitemark evidence was false and that the statistics presented at trial had no factual basis. In addition, using new computer technology to correct for distortion, Richards proved that he could not have been responsible for the bitemark.[5]

The colossal injustices and failures in the U.S. justice criminal system which lead to the conviction of the innocent are becoming ever more apparent. The flippant use of bitemark evidence in the past has been troubling and the continued use of such evidence despite the known problems is worrisome. Defense counsel should critically evaluate any case in which bitemark evidence was involved and there is a potential for innocence. To adequately represent such defendants post-conviction, defense counsel should be cautious to collect all of the original documentation, photos, and notes relied upon by the prosecution's bitemark experts, evaluate the bitemark for possible exclusions, seek DNA testing on any swabs of bitemarks, and talk to the original prosecution experts to see if their position has changed. Further suggestions will be discussed.

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Defense Counsel, Post-Conviction, Wrongful Conviction

G1 A Peculiar Case of a Perineal Injury Miming Sexual Abuse in a Child Run Over by a SUV

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After attending this presentation, attendees will become aware of the occurrence of non-intentional traumatic anogenital injuries in children run over by motor vehicles. These accidents can sometimes conceal the findings of sexual abuses.

This presentation will impact the forensic science community by underlying the necessity of making investigations and carefully putting forward opinions, in a multidisciplinary perspective, led by the strict application of the forensic medical methodology.

A case is described of a suspected pediatric sexual abuse case, involving a nine-year-old female. The girl, while running out of her stepfather's shop, was run over by a slow-moving SUV. The stepfather had a crimal record for prior sexual abuse.

When the ambulance arrived, the little girl was found locked in the shop restroom. The girls mother did not want the doctor to enter. After repeated requests, the doctor entered the restroom where he saw the girl standing with bloodstained panties and trousers. Additional bloodstains were observed in the toilet bowl. Deeply upset and in a state of amnesia, the girl first refused to be seen by the doctor. She relented due to repeated requests and was taken to the first aid hospital in Terni. Multiple excoriations on upper and lower limbs were found by the medical staff who also confirmed the presence of the bloodstains in the panties.

A gynecologist was contacted who, because of the girl's reluctance, decided to continue the exploration under general anesthesia. He described a second-degree perineal laceration between the labial back commissure and the anal sphincter of 2.5cm long which was soon sutured. According to the gynecologist, this injury could be due either to a sexual abuse or post-traumatic injury from the road accident.

The doctors at the hospital reported the fact to the judiciary which, because of the girl's stepfather's criminal records, started a file of documents in the event of a sexual abuse (Art. 609 bis; art. 609 ter, Italian PC). The judiciary entrusted inquiries to the Carabinieri and to the department of forensic medicine in Terni.

The inquiries, at first, strongly indicated sexual abuse and showed that the road accident occurred unexpectedly, while the child was trying to elude her stepfather; however, when questioned by the medical examiner, new witness evidence had been admitted together with an examination of the SUV. Only after these further inquiries did he exclude any hypothesis of violence and declared that the perineal injury was due to the car accident.

In literature, perineal and anogenital injuries are described as the consequence of a car.[1-6] As a matter of fact, these injuries can resemble the ones caused by sexual abuses.

The finding of an anogenital injury in a child can become a diagnostic dilemma in distinguishing a sexual abuse from a non-intentional injury, and medical examiners should always ponder both possibilities, especially if the history is vague or appears unlikely.

A multidisciplinary approach, led by an experienced medical examiner, is fundamental in such cases in order to avoid misdiagnoses. The latter could have devastating results for the children and their families.

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Perineal Injury, Sexual Abuse, Road Accident

G2 A Molecular Approach: Species Composition of the Maggot Mass in Human Cadavers in the Pineywoods Ecoregion of Southeastern Texas

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After attending this presentation, attendees will understand that through collection, extraction, quantification, amplification, and sequencing analysis of the DNA from maggots that colonize a human corpse, the species composition of the maggot mass can be determined. Until now, the maggot mass has been assumed to be of a multiple species composition due to the observation that several species of females will visit the body within a 24-hour period. It was unknown if adult female flies of different species would lay their eggs in the same location on a corpse as other adult female flies. This study tested the hypothesis that a maggot mass is composed of several different species of larval flies.

This presentation will impact the forensic science community by demonstrating how a maggot mass composed of multiple species will bear significant impact on fly developmental studies and growth rate models since the presence of one species can slow or accelerate the development of another species. These developmental rates will thus have a direct effect upon the Postmortem Interval (PMI) estimation. DNA-based methods for identification of maggot species are preferred since the first and second instars of many forensically related maggot species are difficult to identify due to the lack of defining anatomical characteristics and identifying each maggot according to distinguishing morphological characteristics can be time consuming. In addition, the process can suffer from human error if performed by an untrained forensic entomologist.

Depending on time of day and ambient temperatures, adult flies arrive at a human cadaver within minutes to hours after the body has been placed outdoors to decompose. During initial human decomposition, it has been observed that several adult female fly species arrive at the body. Adult female flies arrive gravid and oviposit

immediately. The resulting eggs hatch after a period of time dependent upon the temperature, rate of body decomposition, species of the maggots, and other factors, resulting in the maggot mass. Even closely related carrion species can differ in growth rates, diapauses response, and/or ecological habits. Therefore, accurate identification of an insect specimen is crucial for PMI estimation.

First and second instar maggots were collected from three bodies from September to November 2011. Maggot DNA was extracted using a silica-based method and quantified by real-time PCR. Co-Oxydase enzyme I (COI) and Co-Oxydase enzyme II (COII) gene sequences were amplified by PCR and sequenced. COI and COII are unique markers that are highly conserved because they code for respiratory processes making them species-specific genes. Sequencing products were analyzed by capillary electrophoresis with fluorescent detection. Preliminary data on the second cadaver, through phylogenetic analysis, showed that four first instar samples were related to *Cochliomyia macellaria* and that twelve first instar samples were related to *Phormia regina*. This preliminary data supports the hypothesis that the maggot mass is composed of multiple species.

Forensic Science, DNA Typing, Forensic Entomology

G3 Unusual Work-Related Fatality: Importance of Scene Investigation Combined With Autopsy Findings

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After attending this presentation, attendees will understand the importance of the reconstruction of an unusual, fatal occupational accident by a detailed workplace investigation, combined with the evaluation of circumstantial, radiological, surgical, and, most of all, autopsy findings. The definition of a fatal accident at work adopted by the European Statistics of Accidents at Work project is "accidents at work leading to the death of the victim within a year (after the day) of the accident." In Italy, the main source of information about occupational injuries is the Institute of Insurance for Occupational Illness and Injury (Istituto Nazionale per l'Assicurazione degli Infortuni sul Lavoro; INAIL). According to INAIL, in 2011, 920 Italian workers died from work-related injuries. The majority of the accidental deaths occur either immediately at the time of the accident, or within a few days or a few weeks after the accident.

This presentation will impact the forensic science community by emphasizing how autopsy can play a key role about the reconstruction of the dynamics involved in the occupational event, allowing the identification of any legal responsibilities of the worker or the employers.

A case is presented of a 44-year-old man who was working in a building site, driving a tractor with a rear-mounted flail mower. While he was mowing grass close to a crane, he was struck in his left eye by something propelled by the mower's blades. The victim was rescued by his coworkers and brought to a local emergency department in a comatose state. At the hospital, a CAT scan of his brain revealed a metallic foreign object in the left occipital area, which had penetrated the left eyelid and orbital bone, making a channel into the left fronto-temporo-parietal region. Despite early craniotomy and intensive care, the victim died four days later and organ donation was authorized. Trying to understand the dynamics of the event, a medicolegal autopsy was performed. The external examination of the body showed a 0.6cm wound on the left superior eyelid. A 0.6cm keyhole wound on the superior wall of the orbital bone was also found. During gross examination of the brain, left hemisphere findings included a subdural hematoma, subarachnoid hemorrhage, and intra-axial hematoma. In the occipital lobe, a fragment of a frayed copper cable segment was found which was 4cm in length and 0.3cm in diameter. No alcohol or drugs of abuse were found in the blood, and urine collected at autopsy. The cause of death was identified as diffuse brain damage due to a penetrating head injury.

The workplace investigation showed the presence of a severed electrical cable, partially buried, protruding from the ground, just beneath the mower. The cable revealed the same characteristics as the fragment found in the brain. Therefore, it was thought that, as the man was driving the tractor, the moving mower blade hooked the electrical cable, pulling it out from the ground and severing it. A piece of that cable was projected forward striking the victim's head as he turned to investigate the obstacle interfering with his mowing.

In the case presented, the pre-autopsy reconstruction of the work-related fatality was incomplete because of the lack of detailed information concerning the accident scenario. In fact, it is known that there are many potential contributing factors to work-related accidents including: the nature and duration of the work; type of equipment, tools, or machinery used; the environmental conditions; and, the behavior of the worker. In the majority of cases, the information provided to inspectors and the police by coworkers facilitate a reasonable reconstruction of the occupational accident, but sometimes only the autopsy may detect the real cause of the death and what actually happened to the victim at the scene.

Brain Injury, Occupational Fatality, Workplace Investigation

G4 Iron in the Human Brain: Age-Related Changes and Anatomic Region Specific Differences

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The goal of this presentation is to show the first results of a research work aiming to clarify the role of iron in brain functions, and particularly its implication in neurodegeneration. Since neurodegenerative diseases are strongly age-related and there is a specific region of the central nervous system that seems to be particularly affected in each disease, the main objectives of this research are to study: (1) the changes on iron levels in human brain in relation to age; (2) the regional anatomic differences of iron levels within the brain; and, (3) the differences between individuals with and without evidence of neurodegenerative diseases.

This presentation will impact the forensic science community by giving epidemiology background data concerning iron levels in the healthy human brain. Since no updated and comprehensive data about trace elements in the human brain are available, this study is a relevant scientific contribution for establishing "normal" human brain levels, allowing a comparison with a brain affected by neurodegenerative diseases in an attempt to clarify trace elements' role in the disease process.

The etiology of neurodegenerative diseases is multifactorial, assuming that it involves a complex interaction between natural aging, environmental factors, and genetic predisposition. The involvement of trace elements, particularly iron, in neuronal damage in distinct areas of the brain has been demonstrated in Alzheimer's and Parkinson's disease. However, despite the research work that has been done on the relationship between trace elements and neurodegenerative diseases, the evidence is still fragmentary, and their role remains poorly understood. Most of the current information about the relationship between trace elements and human brain functioning is based on animal studies or relies on determinations in cerebrospinal fluid, blood, and serum. To fulfill this lack, iron levels were quantified directly in human brain tissue in this research. Direct determination of trace elements in samples from patients with neurodegenerative diseases and normal individuals is essential to extend the

understanding of the underlying disease mechanisms, to validate animal models, and to develop therapies that can delay or reverse neurodegeneration processes.

Two groups of individuals submitted to autopsy, performed at the North Branch of the National Institute of Legal Medicine and Forensic Sciences of Portugal, in the first quarter of 2012, were studied. Group A consisted of ten individuals in each age sub-group: 50 – 60; 60 – 70; 70 – 80; 80 – 90 and over 90-years-old. Ten healthy accident victims 20 to 30-years-old were included as controls ("baseline"). Group B consisted of individuals submitted to autopsy with previous diagnosis of Alzheimer's and Parkinson's disease. After verifying all current legal regulations in Portugal for human tissue collection for scientific research purposes, the following areas were sampled from each individual: (1) frontal cortex; (2) superior (2 – A) and medium (2 – B) temporal; (3) basal ganglia including, caudate nucleus (3 – A), putamen (3 – B) and globus pallidus (3 – C); (4) cingulated gyrus; (5) hippocampus; (6) inferior parietal lobule; (7) visual cortex of the occipital lobe; (8) midbrain including the substantia nigra; (9) pons—locus coeruleus; (10) medulla; and, (11) cerebellum—dentate nucleus. Iron levels were determined by Graphite Furnace Atomic Absorption Spectrometry after the microwave-assisted acid digestion of samples.

Results showed that iron distribution in an adult human brain is not homogeneous: the highest levels are found in basal ganglia and the lowest in pons and medulla. In specific areas, iron deposition seems to be age-related since there is a direct correlation between iron levels and age, namely in putamen, cingulated gyrus, visual cortex of the occipital lobe, midbrain, and cerebellum. However, in caudate nucleus and globus pallidus, iron levels seems to decrease with age. In Alzheimer's disease, significantly increased iron levels in basal ganglia were found when compared with healthy people of the same age sub-group.

Aging, Iron Accumulation, Neurodegeneration

G5 Atherosclerosis in Youth — A Study in the North of Portugal

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The goal of this presentation is to study the prevalence and severity of atherosclerosis via autopsies performed on victims aged between 18 and 45 years of age in the North of Portugal, regardless of the cause of death, in order to better understand its impact on the Portuguese population.

This presentation will impact the forensic science community by providing information regarding the prevalence of atherosclerotic vascular disease and how it has been on the rise in developed countries, and the increasingly early onset of acute diseases caused by this phenomenon which may be fatal, particularly acute myocardial infarction or stroke. It is, therefore, essential to discover the prevalence and severity of this disease, especially in the younger population, as in the majority of the cases it is "silent" and the first manifestations of malaise may be sudden death.

International statistics indicate that by 2020, cardiovascular disease, particularly atherosclerosis, will become the leading cause of reduced quality of life due to the resultant disability, morbidity, or sudden death.

Few studies have been performed for this age group in Portugal, and investigations were based on the study of cadavers, being able to determine the prevalence and severity of atherosclerotic disease in young individuals. This will, therefore, give some knowledge as regards the prevalence of atherosclerosis in this age group in Portugal. Another objective is to determine whether results collated with the existing data in international literature of the same subject

In order to assess the extent of disease in the population, vascular tissue samples were collected from all individuals aged 18 to 45 years, regardless of cause of death, who had been autopsied at the Forensic Pathology Service of the North Branch of the National Institute of Legal Medicine.

In each selected case, some of the factors associated with this pathology were also noted: gender, age, smoking, hypertension, obesity, as well as the presence or absence of cardiovascular disease in the family history.

All legal proceedings in Portugal for sampling were observed, and in each case, collection and processing of vascular structures were performed. Particular focus was given to some arteries of larger calibers (ex: aorta, iliac artery, pulmonary artery) with analysis of the type of vascular injury (lipidic striae/atherosclerotic plaque/atheromatous calcification and/or ulceration) to compute a severity score in terms of atherosclerosis.

Arteries of lower calibers responsible for the irrigation of some important organs (ex: middle cerebral artery, coronary artery, renal artery) were also studied. All samples were stained with hematoxylin-eosin and categorized into qualitative classes of increasing severity related to the degree of arterial obstruction utilizing specific software for measuring the vascular percentage of each arterial obstruction (Image J software). The histological examination of the atherosclerotic plaque tissues included the study of its components: presence of calcification, degree of macrophagic infiltration, density of intraplaque vessels, plaque hemorrhage and some of its possible complications, such as thrombosis.

In the majority of the cases studied, manifestations of atherosclerotic disease, including cases in which no risk factors were identified prior to the arterial study were found. In some of the cases, the cause of death was directly associated with the atherosclerotic phenomenon. The results obtained through the study of the arterial specimens in order to better understand the prevalence and severity of atherosclerotic disease will be presented.

This study will also draw attention to an increasingly early onset of cardiovascular disease in the Portuguese population, which will necessitate the implementation of measures involving the control or reduction of some avoidable risk factors, namely those related to lifestyle (eating habits, physical activity, smoking, etc.), particularly in some public facilities like schools or companies.

Atherosclerosis, Sudden Death, Portugal

G6 Utility of Multi-Phase Postmortem Computed Tomography Angiography in Two Lethal Cases of Great Height Falls: A French Experience

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After attending this presentation, attendees will understand the utility of investigating great height fall deaths by postmortem CT angiography.

This presentation will impact the forensic science community by showing how the pre-autopsy postmortem CT angiography can complete the conventional autopsy results and help to understand more precisely the death chronology.

Background: Multi-Slice Computed Tomography (MSCT) has been introduced in forensic sciences in the last decade and the efficacy of this technique has been proven, especially for bone injuries. The use of enhanced MSCT has been recently developed in order to improve detection of vascular injuries and traumatic solid organ injuries.

In the beginning of 2012, the forensic hospital department associated with the University Radiological Center of Toulouse

(France) introduced the use of Multi-Phase Postmortem Computed Tomography Angiography (MPMCTA). Since then, two cases involving falls from significant heights have been studied at the department. The relevant literature was reviewed in order to compare the observed cases with the actual knowledge and to establish the contribution of MPMCTA to the forensic purpose.

Summary of Cases: The first case studied (F1) was that of an 81-year-old woman with known suicidal tendencies. The second case (M2) was that of a 45-year-old man with documented suicidal attempts and previous psychiatric hospitalizations. The estimated heights of fall were about 13 and 12m (42 and 39 ft), respectively.

Methods: For the purposes of MPMCTA, both corpses were prepared with a surgical cannulation of femoral vessels (MAQUET Gmbh & co.KG). The non-enhanced MSCT phase, performed on a 16-units MSCT (Sensation 16, Siemens), facilitated sampling of biological liquids for further toxicological and biochemical analyses. The controlled perfusion device used (Virtango, Fumedica AG) and the injection of paraffin oil mixed to Angiofil® (the contrast agent) allowed acquisitions at three different times: arterial, venous, and dynamic (arterial phase with venous aspiration).

The first radiological view was performed by a Forensic Radiologist, who also performed the autopsies. The second radiologist proceeded to the MDMCTA data analysis without knowing the autopsies' results. Neither of the radiologists knew the radiological reports of the other.

Results: The comparison of MPMCTA-derived data with autopsy reports led to different findings. Most of the pathologic findings, like important cephalic, thoracic, and abdominal injuries, were diagnosed by both techniques. Some diagnoses, observed only by autopsy, included T7-T8 discal disjunction (F1), testicular fractures and contusions (M2). The diagnoses established exclusively with MPMCTA were hemopneumatoceles (F1) and deep bone fractures of fibula's head and a metacarpal bone (M2).

In addition to these classical findings, rare injuries such as double thoracic aortic rupture (F1), right coronary avulsion, and inferior vena cava laceration with right atrium extension (M2) were diagnosed by both techniques.

Discussion: Classical lesions seen in great hight falls have been described in the literature data set for decades. These lesions are mostly due to a deceleration mechanism. All typical lesions were found by both MPMCTA and autopsy; however, there are some subtleties.

The posterior rib fractures, a typical finding in high deceleration lesions, were underestimated during the autopsies. This is due to the difficult access to this anatomical region. It is almost the same for the haemopneumatocele. Indeed, the pulmonary macroscopic dissection collapses the haemopneumatocele, making the diagnosis difficult. On the other hand, some lesions were only found during autopsy. The soft tissues, like the testes, are poorly seen with MSCT, which explains the lack of diagnosis in this case. The discal disjunction has been seen with MPMCTA but was misdiagnosed as a degenerative lesion.

Rare lesions (double aortic rupture and right coronary desinsertion) were clearly individualized by both approaches. Epidemiology and mechanism of aortic traumatic ruptures have been studied by many authors. Depending on the series, aortic lesions due to great height falls represent up to 15% of traumatic aortic injuries. The distinctiveness of our case is the association between an isthmus and descending aortic ruptures. Multifocal aortic lesions, including those involving the descending aorta, are least frequent. Post-traumatic lesions of the right coronary ostium are also rare, with a sparse literature data. The existence of this lesion in our case highlighted torsion and shearing force mechanisms due to the great deceleration velocity. Until now, the few reported cases concerned only object projection or motor vehicle accidents.

Conclusion: The study of these two cases of great height fall deaths using the MPMCTA shows the different injuries related to the high velocity death mechanisms. Compared to the non-enhanced MSCT, MPMCTA has a better sensibility, especially for vascular lesions. Furthermore, this new technique helps in understanding the chronology of injuries before death. For example, the death chronology for M2 was inferior vena cava rupture associated to the right coronary desinsertion, haemopericardium, communication between pericardium and right pleura via a penetrating rib fracture, hemopneumothorax.

CT Angiography, Great Height Falls, Postmortem Imaging

G7 Monolithic Substrate Assisted Micro Liquid-Liquid Extraction of Alprazolam From Urine Samples

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The goal of this presentation is to provide information regarding a new monolithic substrate which was developed for Micro Liquid-Liquid Extraction (MLLE) of alprazolam from urine samples. Under the optimized extraction condition, the new MLLE achieved an extraction recovery of 37% for a 100ng/mL alprazolam-spiked urine sample.

This presentation will impact the forensic science community by discussing microextraction techniques that benefit the chemical analysis by consolidating analytical steps and streamlining analysis. This project presents a newly developed technique that combines the advantages of liquid-phase and solid phase microextraction, while minimizing their common disadvantages.

Even with rapid advances in analytical instrumentation, sample preparation represents the majority of the analysis time and has a significant impact on the analysis outcome. One of the oldest and most prevalent forms of sample preparation is Liquid-Liquid Extraction (LLE). LLE utilizes the properties of solubility and solvent polarity to sequester analytes into an extraction solvent. In traditional LLE, extraction solvent is added to a pH-adjusted sample and mechanically stirred to encourage the analyte to partition into the extraction phase and separate from the matrix components. The extraction phase containing the analyte is isolated, dried, and reconstituted. This technique is time consuming, labor intensive, and relies heavily on the skill of the analyst. In an effort to reduce these factors, MLLE techniques were developed. MLLE techniques use a small amount of extraction solvent (2 to 20µL) to concentrate the analyte from the sample. In MLLE, the analyte is concentrated in microliters of extraction phase, and then the extraction phase is removed and injected directly onto the analytical instrument. The aim of this work was to make a Monolithic Substrate to assist Micro Liquid-Liquid Extraction (MSA-MLLE). The main approach is to synthesize an inert and porous material that is capable of holding microliters of organic extraction solvent in an aqueous environment.

In this work, the monolithic substrate was prepared by sol-gel chemistry. Methyltriethoxysilane (MTES) was found to increase the pore size of resulting siloxane. The addition of bulky alkoxysilanes, such as Phenyltrimethoxysilane (Ph-TMS), to the reaction mixture was shown to contribute to hydrophobicity. The addition of a bulky alkoxysilane increased hydrophobicity by reacting with open hydroxyl groups present on the alkoxysilane. MTES and varying levels of Ph-TMS were chosen as the siloxane precursors. The sol-gel reaction was optimized to a molar ratio of 1:13:4.8:0.1 Methyltriethoxysilane:Methanol:Water:Phenyltrimethoxysilane with the reaction adjusted to pH 10 with ammonium hydroxide. The selected sol-gel produced a large-pored, hydrophobic, and viscous siloxane that could be adhered to a metal surface (a pyrolyzer hook) for MLLE.

The Monolithic Substrate-Coated Hook (MSCH) was used for MLLE. The MSCH appeared porous under magnification and were able to hold organic extraction solvent from 0.3 to 1.4µL of toluene prior to exposure to water. After 20 minutes of exposure to stirred water, the retained solvent dropped to 0.1 to 0.2µL depending on the molar ratio of Ph-TMS. Alprazolam is used as a test drug in this

experiment because it is commonly encountered in toxicological analysis. It is also readily available and has been studied by using other microextraction techniques. One of the MSCHs was able to concentrate alprazolam from a spiked urine sample using both toluene and octanol as extraction solvents. The best condition for MSA-MLLE was extraction with octanol for 20 minutes with 600rpm stirring. This condition achieved an extraction recovery of 37% for the low spike (100 ng/mL) and 7% for the high spike (500ng/mL). Unfortunately, the MSCH could be thermally degraded after 5 – 10 runs using thermal desorption. Increase the thermo-stability of the MSCH should be addressed for future work.

Forensic Science, Alprazolam, Extraction

G8 Deaths Due to Blast Effects of Electrocution: A Case Series

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The goals of this presentation are to discuss the dangers of electrocution, the blast effects of electrocution, circumstances of hazardous exposure and the possible prevention of the hazard through the cases done in India. The attendees will be briefed on the mechanism of electrocution deaths with the case history and all the relevant details with detailed description of injuries supported with photographs. Discussion will be also extended to the pathology of electric shock accompanied with histology of cases.

This presentation will impact the forensic science community by sensitizing them to the issue of deaths due to electrocution, especially the blast effects of electrocution. The demarcation line between the injuries caused by electrocution and lightning runs very thin when it comes to differentiating them, which needs to be probed. As the severity of the electrical injury depends on the pathway of the electric current, it becomes vital to determine how the injury occurred. The autopsy findings of the cases will be highlighted along with supporting photographs and histology slides, concluding that there is no specific therapy for electrical injuries and that's the reason to believe that prevention remains the best way to reduce morbidity and mortality due to electrocution.

Death caused by electric shock is simply referred to as electrocution. Despite widespread and extensive usage of electricity for household as well as industrial purposes, the proportion of deaths due to electric shock is meager. Gross/visible damage due to electrocution may range from nil to extreme. Many factors, related both to victim and environment, play a role in determining the effects of electrocution. Death can be instantaneous due to cardiac fibrillation, respiratory arrest, or electro-thermal injuries caused by heat generated by the current. The heat, thus generated within the body in the latter situation, may cause explosive injuries, including amputation or rupture of organs themselves. Blast effects on the victim are more commonly noted in cases of lightning injuries where the lesion is attributed to direct current flow or due to a secondary fall after being struck.

Three different cases with blast effects of electrocution which were not witnessed and, hence, created undue anxiety for the investigating agencies, the victims' families, as well as the general public regarding the manner of death in those victims will be presented. Doubts were raised due to atypical and bizarre distribution as well as patterns of injuries on the victims. Electrocution deaths are mostly accidental, rarely suicidal, and more rarely homicidal. It becomes important to thoroughly investigate and document the circumstances of electrocution for insurance claims and to take precautions in relation to safety measures. History, in these cases, was insufficient. The deaths occurred instantaneously, and, hence it, was challenging for the forensic doctors to co-relate various findings. The findings at autopsy mimicked those of blast injuries due to lightning or explosives, but careful documentation and interpretation, along with the crime scene investigation, clinched the diagnosis.

Electric Shock, Blast Injuries, Autopsy

G9 Sudden Death by Occult Metastatic Carcinoma

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After attending this presentation, attendees will learn about an unusual cause of natural sudden death from pulmonary hypertension as a result of an unusual pattern of pulmonary metastasis from an occult gastric adenocarcinoma.

This presentation will impact the forensic science community by illustrating a rare but important presentation of occult metastatic carcinoma which led to sudden death in a young patient. The objective is to inform forensic pathologists and medicolegal investigators of this bizarre mechanism and cause of death that can only be discovered through careful autopsy.

A 33-year-old Filipina female, with no significant past medical history, was admitted to the emergency room with a several-month history of a cough. She reported taking antibiotics without relief. Imaging revealed slight coarsening of the interstitial markings and no evidence of a pulmonary embolism. Laboratory values were remarkable for an elevated D-dimer (>5000). She was discharged from the emergency room with prescriptions for albuterol inhaler and oral prednisone. One month later, she returned to the hospital with a two-week history of abdominal pain, nausea, vomiting, dyspnea, lightheadedness, and chest pain. The CT was again negative for pulmonary embolus, showing bilateral infiltrates in her upper lung lobes. Her white blood count at that time was elevated to 15K/uL. She was admitted for presumed pneumonia; however, a rapid response was called within hours of admission due to an acute hypoxic episode and subsequent syncope when she tried to get to the bed from the bathroom. After approximately two hours of advanced cardiac life support, the patient expired.

Autopsy revealed an adult female with gross edema and scattered purpura from terminal resuscitation efforts but no significant trauma. Internal examination revealed a thickened stomach wall which, on microscopic examination, was infiltrated by poorly differentiated adenocarcinoma in a linitus-plastica type pattern. Grossly, multiple abdominal lymph nodes were involved by metastatic tumor. Immunohistochemical staining was strongly positive for CK7 and CK20, supporting the diagnosis of a primary gastric carcinoma.

The lungs were notable for fine reticular nodular densities bilaterally. Microscopic examination showed a tumor infiltrate involving the microvasculature, significant arteriolar fibrointimal hyperplasia, and interlobular septal lymphangitic spread. These findings are consistent with Pulmonary Tumor Thrombotic Microangiopathy (PTTM) with the additional finding of extensive extravascular compression by tumor cells.

PTTM is a well-described complication in patients with adenocarcinoma. The typical presentation involves acute pulmonary hypertension (as evidenced by progressive dyspnea, hypoxemia, cough, and hemoptysis), progression to right-sided heart failure and sudden death, often before the adenocarcinoma is discovered. It has been reported in 3% of patients who die with adenocarcinoma (breast, prostate, lung, pancreas) and 25% in patients who die with gastric adenocarcinoma; however, only 8% of patients with tumor emboli experience morbidity or mortality. The diagnosis of tumor emboli is typically not made until postmortem examination. Patients with PTTM develop rapidly progressive pulmonary hypertension secondary to tumor cells occupying a large proportion of small pulmonary arterioles. This in turn leads to fibrointimal hyperplasia and a significant

reduction in the pulmonary vasculature available for gas exchange. The pathophysiology of PTTM remains elusive. It has been suggested that carcinoma cells may produce certain substances that influence the surrounding pulmonary vasculature.

Histologically, medium-sized peripheral pulmonary arteries and smaller arterioles are involved with acute and organizing platelet-fibrin thrombi, small artery intimal fibrosis, and adjacent intralymphatic tumor. These elements do not cause simple mechanical obstruction of the affected vessels. By adhering to the vascular endothelium, they are thought to activate the coagulation cascade and release inflammatory mediators, thus resulting in the formation of microthrombi, stimulation of subintimal proliferation, and smooth muscle colonization of these lesions. The result is a diffuse narrowing of the pulmonary arteriolar system, increased vascular resistance, and marked secondary pulmonary hypertension. The patient had both the classic clinical and histologic features of PTTM with the additional prominent feature of extravascular compression by intralymphatic tumor cells. These features undoubtedly caused her precipitous decline and the mechanism of death in this case is lethal pulmonary hypertension induced by underlying adenocarcinoma.

In the practice of forensic pathology, PTTM should be considered and recognized as a potential cause of pulmonary hypertension. Medical examiners must, therefore, search for occult malignancy via autopsy in order to identify or exclude metastatic cancer as the underlying cause of death.

Thrombotic, Microangiopathy, Adenocarcinoma

G10 A Clinicopathological Correlation of Non-Compaction Cardiomyopathy

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After attending this presentation, attendees will learn about the entity of Non-compaction Cardiomyopathy (NCCM) and how to determine or confirm the diagnosis through postmortem examination.

This presentation will impact the forensic science community by discussing that even though non-compaction cardiomyopathy is uncommon, forensic pathologists should be familiar with this abnormality, not only because it can lead to heart failure, arrhythmias, and embolic events, but also because it demonstrates an increased frequency of familial occurrence.[1]

The deceased was a 46-year-old woman with a history significant for cardiomyopathy, cardiac arrhythmias, congestive heart failure, and alcoholism. An echocardiogram performed two years prior revealed heavy trabeculation consistent with a myocardial NCCM. She suffered from recurrent atrial tachyarrhythmias, including atrial fibrillation with rapid ventricular response, that was refractory to multiple ablation procedures.

While attending a regularly scheduled appointment with her cardiologist, the patient developed tachypnea at rest and atrial fibrillation. She was immediately transferred to the cardiac care unit where she developed increasing dyspnea at rest, productive cough, and lower extremity edema. Chest auscultation revealed atrial fibrillation and bibasilar crackles. Electrocardiogram findings were negative for myocardial infarction. Two days later, the patient spontaneously converted to a normal cardiac rhythm, and she was put on sotalol for rhythm control. A few hours later, the patient experienced a torsades de pointes arrhythmia, a potential complication of sotalol therapy. A successful code was performed; however, the patient aspirated at the time of intubation. Following the code, she remained persistently hypotensive with hypoxemia refractory to medical treatment. Her chest roentgenogram showed extensive consolidation of both lungs. Clinically, the patient remained unresponsive with fixed and dilated pupils. Given the poor prognosis, care was withdrawn and the patient expired.

Autopsy revealed a globally enlarged and dilated heart weighing 424g, which was significantly greater than the sex and body mass adjusted mean of 251g (expected range 171g – 368g), a finding consistent with her history of congestive heart failure. Cross sections of the heart revealed an extensive amount of non-compacted myocardium in the left ventricle, which was most prominent in the distal half and on the lateral wall of the left ventricle. The ratio of non-compacted-to-compacted myocardium was 4.3:1. These findings supported the antemortem diagnosis of NCCM. Microscopically, increased amounts of non-compacted myocardium were seen with variable fibrosis within the non-compacted myocardium. No acute changes were seen. Additionally, the foramen ovale was patent (1.2cm), and multiple old intraparenchymal brain infarcts were identified. The etiology of these infarcts could not be determined with certainty; it is possible that they may have been embolic in origin and associated with the NCCM and arrhythmias and/or associated with the patient's patent foramen ovale.

NCCM is a rare myocardial abnormality with an unclear prevalence. Several studies have determined a prevalence of 0.05% – 0.25%.[1] The age at presentation is highly variable. The cardiac manifestations include heart failure, arrhythmias (atrial and ventricular), and embolic events, all of which were seen in the deceased.[1] In most cases, it is a congenital abnormality due to the arrest of normal embryogenesis of the endocardium and myocardium and is often associated with other congenital cardiac malformations.[2] Familial and sporadic forms of NCCM have been described with both X-linked and autosomal patterns of inheritance.[3]

The mechanism of death in the case presented was aspiration pneumonia secondary to complications of intubation at the time of cardiac arrest. The cardiac arrest was initiated by a torsades de pointes arrhythmia which was most likely induced by the sotalol therapy. NCCM should, therefore, be deemed the underlying cause of death since the intended purpose of the sotalol therapy was to manage the atrial arrhythmia, which is a known complication of NCCM.

In the practice of forensic pathology, NCCM should be considered and recognized as a potential cause of heart failure, fatal arrhythmia, and embolic events including cerebrovascular accidents. Medical examiners must be especially aware of this anomaly since it has been recommended that surviving first-degree relatives be screened by echocardiography given the increased familial association.[2,4]

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Noncompaction, Cardiomyopathy, Left Ventricle

G11 Delayed Death Following Penetrating Stab Wound to the Heart

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After attending this presentation, the attendees will: (1) better understand characteristics of delayed cardiac tamponade, as described in a case presenting for medicolegal autopsy in which the

medical examiner determined that death resulted from a homicidal stab wound three weeks following the event; (2) evaluate the role of medical records, police report, and time course in determining the cause and manner of death in such cases; and, (3) evaluate impact of such characterization of cause and manner of death on courtroom testimony.

This presentation will impact the forensic science community by utilizing a case presentation with literature review to evaluate the common factors which should be present in order to assign a remote injury a role in certification of medicolegal death.

A 24-year-old man was stabbed in the left lateral chest during an altercation three weeks prior to his death. He was admitted to the hospital for treatment. A computed tomography scan on the day of injury showed a mild pericardial effusion with pneumopericardium and a moderate left hemothorax. An area of linear hyperenhancement was noted near the left ventricular apex. A chest tube was placed and drained a large (800mL) left hemothorax. Follow-up X-rays showed normal mediastinal contours and cardiac silhouette with resolution of the hemothorax. The patient remained stable and was released from the hospital after three days and did not seek any follow-up care.

After a symptom-free interval of three weeks, he was brought back to the hospital by EMS complaining of sudden-onset, left-sided chest pain and syncope. He stated that he “had to take a knee” and said, “I feel like there is air in my lungs.” While being evaluated, he deteriorated suddenly and an emergency intubation was performed. Ultrasound revealed a large pericardial effusion. 30mL blood was removed via pericardiocentesis. Due to persistent pericardial effusion, thoracotomy was performed with the evacuation of an additional 150mL of mixed clot and fresh blood. Despite these interventions, the patient could not be resuscitated.

At autopsy, the decedent was noted to be well-developed and muscular. Multiple fresh puncture wounds of the subxiphoid region, a nine-inch stapled thoracotomy incision, and a one-inch healing linear scar with evidence of suturing (believed to the site of chest tube placement during initial hospitalization) were noted. A one and one quarter-inch obliquely oriented healing linear scar was present on the skin of the left chest just below the sixth rib and slightly anterior to the mid-axillary line.

On internal examination, there was 500mL bloody left pleural fluid, an additional 500mL bloody peritoneal fluid, and abundant clot within the pericardial sac, left pleural cavity, and peritoneal cavity. Indistinct fibrous scarring marked the site of injury on the parietal pleura. Areas of scarring (likely healed injuries) were noted in the lingular portion of the left lung. A one-quarter-inch defect with surrounding hemorrhage was present in the pericardium overlying the apical portion of the anterior left ventricle.

There was a large area of epicardial hemorrhage over the apex of the heart. A half-inch-in-diameter defect of the anterior apical left ventricular myocardium was identified underlying the pericardial defect. Surrounding the defect was a zone of softened and hemorrhagic myocardium measuring up to one inch in diameter. Upon sectioning, it was revealed that the defect perforated the left ventricular wall. Microscopic sections of the injured myocardium showed extensive hemorrhage and necrosis with evidence of chronicity (hemosiderin deposition and fibroblast proliferation).

A large percentage of patients with stab wounds to the heart, between 80% and 90%, develop acute cardiac tamponade. Delayed cardiac tamponade, however, is a rare complication of stab wounds to the chest, with delays of up to 100 days between injury and development of tamponade reported. The causes of delayed cardiac tamponade vary and may include displacement of thrombus from a cardiac mural wound, tearing of adhesions formed during wound healing, or pericarditis due to postcardiac injury syndrome.

It is believed that the initial injury to this decedent’s heart penetrated, but did not perforate, the left ventricular wall and that the injury resulted in infarction and necrosis leading to a weakening of the heart wall with subsequent perforation and tamponade.

Tamponade, Stab, Heart

G12 Usability of RNA Purified From Forensic Pathology Tissue Samples for Molecular Investigations: The Effect of Tissue Decay on RNA Quality

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After attending this presentation, attendees will learn more about the quality and integrity of RNA extracted from human tissue samples with variable degree of tissue decomposition, and its usability for molecular research. Knowledge about the postmortem stability of RNA and usability of RNA, purified from forensic samples with variable degree of tissue decomposition, can contribute to resolve numerous causes of unexpected deaths. The molecular RNA investigations, such as gene expression analyses, can be a usable supplement to routinely perform histopathological and toxicological investigations.

This presentation will impact the forensic science community by improving the knowledge about the influence of postmortem degradation of RNA due to decomposition of tissue on the results of molecular analyses based on Polymerase Chain Reaction (PCR) in order to support the growing interest in applying molecular analysis in forensic pathology.

RNA is generally considered to be more unstable than DNA, and its usability in PCR-based analysis is more challenged. The goal of this study is to perform a systematic investigation of how body decomposition affects the quality and usability of RNA extracted from muscle tissue samples taken at forensic autopsies and from the corresponding samples of muscle undergoing the standard procedure of fixation in formaldehyde and embedding in paraffin (FFPE).

The degree of decomposition was based on postmortem interval in bodies stored under similar conditions. In order to study the influence of storing condition at low temperature, a group of bodies which were found in water, which is normally cold in Denmark, were identified.

Using the internal autopsy database; random samples of 40 bodies of adults who were found inside at room temperature and had different degrees of body decomposition ranging from no sign of body decomposition to severe body decomposition with mummification and sceletation prior to autopsy, were identified. Moreover, nine bodies were identified that had been missing at least two weeks and were found in the water. The cases were routinely autopsied at the Department of Forensic Medicine University of Aarhus between 2009 – 2011. The study samples were stratified into five groups according to postmortem interval and storing condition.

RNA from unfixed muscle samples was isolated by organic extraction and isopropanol precipitation. RNA from FFPE muscle was isolated using the commercially available kit (Roch). Complementary DNA (cDNA) was synthesized by using the RT-for-PCR kit cDNA synthesis kit with the use of random hexamer primers. The real time PCR assay targeted an 86 basepair amplicon of the human ACADM gene. The thermal cycling consisted of 15 min at 95°C followed by 50 cycles of 30 s at 95°C and 1 min at 60°C with collection of fluorescent data at 60°C.

It was found that the RNA from unfixed samples yield, quality, and ability to support RT-PCR amplification though decreasing with increasing decay of the source tissue. Interestingly, it was found that the ACADM transcript was detectable in almost all samples with the least decay (until one week after death). In contrast, RT-PCR amplification of the ACADM transcript was only possible for one sample with the high degree of decay with a quantification cycle of 38 (indicating low abundance). Furthermore, the ACADM transcript was amplifiable in all RNA samples from tissue derived from bodies found in water, thus, supporting that RNA molecules are well-preserved within water-immersed bodies. The only RNA, purified from FFPE muscle samples taken from bodies with no decay (one to three days

after death), were able to support RT-PCR amplification of the ACADM transcript.

The data supports that the standard FFPE procedure increases degradation of RNA. RNA transcripts from unfixed muscle samples often were detectable in muscle tissue up to one week postmortem that may stimulate further RNA-based molecular analysis in forensic science.

Forensic Pathology, RNA Recovery, Autopsy

G13 On the Effects of Preservation, Blade Angle, and Intra- and Inter-Individual Differences on the Identification of Tool Class Characteristics Retained on Human Costal Cartilage in Cut Marks Analysis

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After attending this presentation, attendees will understand how the preservation method, knife blade angle, and intra- and inter-individual differences in human cartilage samples affect the ability of costal cartilage to retain the original class characteristics of the knife, as measured by the distance between consecutive striations in cut mark analysis.

This presentation will impact the forensic science community by contributing to the identification of tool-class characteristics with new data concerning the analysis of cut marks on human costal cartilage which is a valuable source of data to forensic scientists. Human tissue has seldom been utilized in experimental studies, although its structure and biomechanical characteristics differ from that of non-humans, thus challenging the interpretation of the results acquired from animal models in order to extrapolate them to a human model.

Materials and Methods: The 160 cartilaginous samples used in this study originated from the ribcage of seven male cadavers that underwent autopsy at the North Branch of the National Institute of Legal Medicine in Portugal. Three different serrated knives were purchased from a large department store to be used in the study. Initially, 40 samples of dissected costal cartilage were manually cut using the same knife following a motion parallel to the long axis of the teeth in the serrated edge. Casts of the "fresh" cut surface were made using casting material for forensic use. The 40 samples of the dissected costal cartilage were then placed in a formalin 10% solution for seven days after which were all re-casted. Both "fresh" and "preserved" casts from each of the 40 samples were observed and photographed. The "fresh" casts images were then compared with the "preserved" casts images by direct image superimposition. In the second phase of the study, a total 120 samples of costal cartilage were used. Samples from two individuals were assigned to each knife. Each individual provided 20 cartilage samples. Cartilage samples were manually cut using each of the three knives following two motions, one parallel and one perpendicular to the blade's teeth long axis. Casts of the samples were made with a casting material for forensic use. Image capture and processing were performed with a stereomicroscope and its software. Direct image superimposition was used to test how the preservation method used for the cartilage samples (formalin 10%) affects preservation of cut marks on the cartilage surface. The distance between striations in the acquired image was measured and data was statistically analyzed.

Results and Conclusions: Assessing the influence of formalin preservation on the striation pattern, direct and complete image superimposition, when comparing the two groups of casts ("fresh casts" vs. "preserved casts") showed that no significant distortion or shrinkage of the striation pattern occurred by preservation of the cartilage samples in a 10% formalin solution for seven days. The blade's penetration angle and the inter-individual differences were shown to affect the identification of the tool class characteristics from the striation pattern observed in a kerf wall, although this fact seems to be related only to the degree of calcification of the costal cartilage. Intra-individual differences do not seem to be relevant enough as to affect in a significant way the identification of the tool class characteristics from the striation pattern observed in a kerf wall for the same knife following the same motion. The degree of calcification of the cartilage is a source of great variation regarding the interpretation of striations pattern in cartilage.

Cut Marks, Tool Marks, Knives

G14 A Method for the Separation and Isolation of Intact Single Cells From Paraffin-Embedded, Formalin-Fixed Tissue Using Laser-Dissection Microscopy

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After attending this presentation, attendees will gain an understanding of the methods used to separate, identify, and isolate single cells from paraffin-embedded tissue blocks for the purposes of single-cell studies or assays, as well as the specific methodologies employing laser-dissection microscopy, and the success rate using this method in capturing single cells.

This presentation will impact the forensic science community by providing results from replicate experiments in an area with applications to histology, pathology, DNA analysis, and molecular genetics. This presentation will add to research being carried out in forensics by demonstrating a reliable process by which single cells from archival paraffin-embedded tissues can be isolated for the purposes of single-cell assays.

The separation and isolation of single cells from formalin-fixed, paraffinized tissue was necessary in a recent study into the effects of mitochondrial heteroplasmy at a cellular level. While the results from the study were inconclusive due to an issue in the SNP primer design, the methodology for the removal of paraffin, the mashing of the tissue to free cells, and the use of laser-dissection microscopy was seen to be highly effective at the isolation of intact single cells from a previously formalin-fixed and paraffinized tissue source. The use of laser-dissection microscopy is a commonly accepted approach for cell isolation along with flow cytometry and the use of optical tweezers.[1-3]

The cells were freed from the tissue using a combination of heated water washes to melt the paraffin, followed by the mashing of the tissue block between the frosted ends of microscope slides. The frosted glass acted as a grinding surface to release cells from the intact tissue, while the remaining space between the slides allowed the lateral migration of the intact cells without further mechanical breakdown of the cell structure. The mashed tissue and cells were then sieved to separate the cells from the gross tissue, after which the cells were counter-stained with a combination of a mitochondrial-specific fluorescent dye (MitoTracker® Green FM) and Nuclear Fast Red and then affixed to the laser dissection microscope slides.

The use of laser-microscopy on such cells following separation and fluorescent and optical staining was critical for the proper identification of intact single cells. The methodology used in the process of laser dissection was highly controlled to ensure the proper isolation of a single cell, which was shown to be highly reproducible.

To ensure collection, the cells were ablated from the microscope slide and into a collection buffer ensconced within the one ml collection tube cap. The capture buffer was not only a support medium to trap the isolated cell, but also contained the appropriate volume of amplification buffer to send the single cell directly to a one-step amplification which simultaneously lysed the cell and amplified the cellular mtDNA. The resulting detection of the SNP base in question demonstrated an overall collection efficiency of 94.6%, with 681 out of 720 collected cells yielding a signal upon fluorescent detection.

The results of the study demonstrated that the methodology for separating and isolating the cells from the paraffinized tissue was reliable and reproducible. The use of the laser dissection microscopy was effective under the controlled conditions for collecting single cells and was highly specific for single cells selected on a slide when other cells and cellular debris were present within the same viewing field. The confirmation of the single cells was based on the downstream testing and resolution of the SNP base from the mtDNA, which confirmed not only the presence of a captured cell but the presence of only one cell compared to clusters of cells.

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Isolation, Single-Cells, Laser Dissection

G15 The Importance of Microscopic Examination of the Lungs in Decedents With Sustained Central Intravascular Catheters

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After attending this presentation, attendees will understand the pulmonary vascular complications of intravenous injection of oral medications and appreciate the frequency of fatal misuse of prescribed long-term vascular access devices, such as central venous lines and peripherally inserted central catheters. Attendees will gain insight into the need for microscopic examination of lung tissue with polarized light and be able to accurately identify, characterize, and diagnose various states of disease microscopically. In addition, the attendees will gain an understanding of some common indications and complications for prescribed placement of long-term vascular access devices.

This presentation will impact the forensic science community by demonstrating the frequency of fatal intravenous injection of oral medications in people with indwelling central intravascular catheters. By highlighting the importance of microscopic examination with polarization of lung tissue in all patients with central intravascular catheters, these findings may directly affect the cause and manner of death in individuals with numerous medical comorbidities.

Recreational intravenous drug users may choose to inject suspensions of oral medications into peripheral veins, muscle, or subcutis using a hypodermic needle. However, central lines, peripherally inserted central catheters (PICCs), implanted vascular ports, and hemodialysis catheters may provide convenient vascular access without the stigmata of other methods. Pulmonary vascular lesions including microemboli, vascular wall and interstitial granulomas, and eventual pulmonary vascular bed destruction frequently result. Herein, the a seven-case series is presented of forensic autopsies performed during the last five years at the Medical University of South Carolina where the decedents all had pulmonary vascular complications stemming from intravenous administration of oral medications through a residing central venous line, peripherally inserted central catheter, vascular access port, or hemodialysis catheter. All of the decedents were in their fourth decade of life; two were male and five were female. Comorbidities that resulted in central venous line, hemodialysis catheter, or vascular access port placement will be delineated and discussed, and concurrent toxicology results will be reviewed. The frequency of these cases and the misuse of medically prescribed long-term vascular access catheters require particular attention. An autopsy of an individual with a vascular access device should include microscopic examination of the lungs under polarized light to identify the fillers and binders used in the production of the oral medications. These insoluble, birefringent substances include compounds such as magnesium trisilicate (talc), microcrystalline cellulose, corn starch, and potato starch. The location of such material should be documented, and long-term effects, such as foreign body granulomas and vascular changes, should be described. Massive embolization of foreign material evident in the pulmonary vasculature explains sudden death in cases where acute drug toxicity may not. Chronic findings including granulomatous obliteration of the pulmonary vasculature with the resulting pulmonary hypertension and right heart failure may explain a cardiogenic death due to intravenous drug use. Therefore, without such investigation, the cause and manner of death may be inaccurately ascribed to the decedent's comorbidities, or the contribution of pulmonary vascular complications stemming from intravenous administration of oral medications may not be appreciated.

Central Lines, Drug Use, Lung Microscopy

G16 Sudden Death From Aggressive Pansinusitis and Pituitary Abscess With Clinical Features Suspicious for Intracranial Trauma

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After attending this presentation, attendees will be aware that severe pansinusitis can lead to pituitary abscesses that can be rapidly fatal and have clinical features suggestive of intracranial trauma.

This presentation will impact the forensic science community by describing a rare, fulminant natural disease process that was initially suspicious for trauma.

Pituitary abscesses are rare lesions that are usually confined to the pituitary gland and can be found arising from other pituitary lesions. They can infrequently occur from direct extension of severe or chronic bacterial and fungal sinusitis. They are usually associated with chronic or indolent headaches and facial pain that leads the patient to seek treatment, but they can be rapidly progressive with systemic manifestations resulting in sudden death.

Case Description: The decedent was a 38-year-old male with a past medical history of sciatica and morbid obesity who was found unconscious at home by a co-worker after not appearing for work. He had not complained of any headache, facial pain, or ear pain the night prior; however, he had consumed a large amount of alcohol and taken prescription pain medication that night.

After transportation to the hospital, traumatic head injury was suspected because of diffuse soft tissue swelling involving the left side of his face and head and rust-colored drainage from his left ear and nose. A Computer Tomography (CT) scan of the head found no overt trauma but did show opacification of the left external auditory canal associated with marked soft tissue edema. The paranasal sinuses also had mucosal thickening, mild opacification, and air fluid levels. A Magnetic Resonance Imaging (MRI) scan performed a few hours later showed extensive soft tissue edema and newly developed

opacification of the bilateral mastoids and middle ear spaces. There was also a marked increase of the paranasal sinus opacification, mucosal thickening, and fluid levels in comparison to the earlier CT images. The opacification in the posterior sphenoid sinus abutted the anterior sella turcica with focal destruction of the bone. Diffuse cerebral edema with cerebellar tonsillar herniation and global hypoxic-ischemic injury was identified. Although his condition stabilized, he had suffered a global cerebral insult eventually culminating in brain death.

The case was referred to the medical examiner due to continued concerns about possible trauma because of the rust-colored drainage being suspicious for possible blood issuing from the left ear and nasal passages and because of the acute decompensation.

The major findings at autopsy included thick yellow-pink purulent discharge from the left ear and nares. The left side of the face and head had diffuse soft tissue edema, and an eight by six centimeter (cm) erythematous and purulent ulceration involved the left preauricular area with extension into the external ear canal. The sphenoid bone was dissected to reveal purulent, mucoid material filling the paranasal sinuses consistent with pansinusitis. The purulence and necrotic debris extended into the sella turcica raising the concern of a pituitary abscess. This was confirmed histologically by acute inflammation and marked necrosis involving the majority of the pituitary gland. Thin tan-yellow purulent fluid drained from the middle ear when the petrous portion of the left temporal bone was removed. Multiple bacterial and fungal cultures were performed but did not isolate a causative organism. No intracranial trauma was present.

The autopsy was able to confirm that the decedent suffered from a rare, fatal, natural disease consistent with rapidly progressing pansinusitis leading to a pituitary abscess and severe sepsis. This case illustrates the importance of pituitary examination at autopsy, particularly in the setting of sudden death and severe sinusitis.

Pituitary Abscess, Sinusitis, Sudden Death

G17 Child Homicides in Adana, Turkey

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After attending this presentation, attendees will learn the incidence of child homicides in Adana, Turkey, from January of 2009 to December of 2011, and its comparison to larger studies

This presentation will impact the forensic science community by increasing awareness, which weapons are mostly used in childhood homicides, the most common age groups of homicides, and if there is any relationship between the weapons used and age groups.

Violence is a common and important public health problem in Turkey, as it is all over the world.[1,2] Homicide is the last point of no return and solution. In nearly all communities, homicide is being accepted as a most serious crime, and it is being punished with severe sanctions while prevention strategies are being developed. It was said that violence-caused deaths (homicide and suicide) induce more than 50,000 people's deaths between the ages of 15-24 every year in the U.S. with homicide being the second most common reson of death.[3]

According to the United Nations Convention on the Rights of the Child, all human beings below the age of 18 are considered children, except for those who have previously attained majority.[4] According to the same convention, contracting countries are obligated to make a maximum effort for the survival and development of children. They are obligated to take precautions and make legal arrangements, correcting incommodities of their laws due to the necessity of convention. Governors must know that childhood deaths, which are caused by violence, are preventable only by taking precautions.

For this study, 5,159 cases were investigated retrospectively and were medicolegally autopsied at the Institute of Forensic Medicine Adana Group Authority Morgue Specialty office between the years of 2009 and 2011. One hundred twenty cases below the age of 18, which were determined to be homicides according to prosecution records, crime scene investigation reports, and autopsy signs, were used in the study. Cases were investigated according to cases' ages, genders, objects (weapons) causing death, and localization of wounds. Toxicological analyses of all cases were reviewed.

It was seen that 79 (66%) of 120 child victims were male and 41 (34%) were female. The youngest was 7-month-old and the eldest 18-years-old. The average age was 13.5 years. It was detected that, when the children were grouped according to their ages, most cases 85 (70.8%) were between the ages of 13 and 18. Looking at methods of homicide, it was seen that firearms were in first place with 73 (60.8%) cases, sharp objects followed with 28 cases, and obtuse traumatic lesions were in third with 14 cases. Looking at injured parts of the victims, it was detected that the head was in first place with 50 (41.7%) of the cases, followed by the rib cage area with 21 cases. It was seen that the most wounds by firearms in a single case was six and the most penetrating stab wounds in a single case was 33. It was detected that, when looking at shooting ranges in the cases killed by firearms, the shooting was done from an adjacent range in 40 cases. Tetrahydro Cannabinoid (THC) was found in three cases and amphetamine was found in one case per toxicological reports.

This study was done with the goal of revealing the incidence of childhood homicides at Adana with the goal of calling the attention of governors whose charge was protecting the children exposed to that violence. It also provides data needed for fulfilling the responsibilities of governors according to the international conventions which they were signed.

Even if removing the violence isn't possible, revealing its reasons and methods provides the possibility of forming strategies. In our study, it was seen that, like most developed and developing countries, homicides by firearms is in first place. But it was seen that in some countries, where the firearms' usage is seriously limited, penetrating stab wounds take first place. Limiting the access to firearms will lead to a decline in childhood homicides in all age groups.

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Child, Homicide, Teenage

G18 Homicide or Suicide? An Unusual Case of "Spiritual" Death

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After attending this presentation, attendees will be able to understand that crimes with spiritual aspects are unlikely to be interpreted. The crime scene investigation and autopsy data with histological, biological results and circumstantial elements are basic to reconstruct a crime hypothesis.

This presentation will impact the forensic science community by demonstrating that making a possible link between religion, spiritualism, and manner of death is not easy. Durkheim in 1897 published one of the most important studies about the role of religion in suicide. After this report, many authors presented cases and statistical reports about crimes with spiritual backgrounds.

A case of a 36-year-old man who, after being missing for one day, was found in a cave close to an olive grove will be presented. According to the parents' story, shortly after reading a book titled

Celestino's Prophecy, the victim showed a state of spiritual delirium characterized by the intention to "purify the world" and to "free the world from evil and technology." He left the house, saying to his wife and sons that he "would return only with the spirit."

The crime scene examination revealed the body was found supine, wearing only underpants. A hammer and a hacksaw were found less than one meter to the right and above his right hand.

The external examination of the body showed diffuse, numerous yellowish-red abrasive lesions due to the action of the local microfauna (ant activity). The frontal region of the head displayed a 23-centimeter (cm) lacerated wound extending to the occipital area. Below this injury, two parallel, longitudinal lesions of 17cm and 10cm with large hemorrhagic infiltration of the periosteum were found. The brain tissue showed one cut injury (5cm long and 2cm deep) with surrounding hemorrhagic infiltration. Near major cuts were numerous longitudinal and oblique scratches (hesitation marks). Histological examination confirmed macroscopic evidence.

No alcohol or drugs of abuse were found in the blood or urine collected at autopsy. The cause of death was identified as diffuse hemorrhage and brain damage caused by multiple blunt and sharp force head injuries.

Crime scene investigations showed the presence of numerous blood spots on the cave walls and floor with a large pool of blood about a meter from the head of the victim. In the surrounding area 16 olive trees were found whose trunks had bloodstains that genetic analysis showed belonged to the victim. Regarding the manner of death, the victim banged his head on each of 16 olive trees; thereafter, the head was cut twice. It is difficult to say if someone was present at the moment of his death.

The case was closed as homicide (spiritual/satanic homicide?) by unknown, despite doubts about the possibility of the case being a very unusual suicide. Crime scene investigation and successive genetic analysis did not reveal the presence of another person on the scene; on the other hand, only a great alteration of the victim's behavior (mystic delirium?) could explain a suicide with such head injuries.

Spiritual Crime, Traumatic Brain Injury, Crime Scene Investigation

G19 Sudden Unexpected Death in a Pregnant Young Woman at 23w+6 Hospitalized for Kidney Infection

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After attending this presentation, attendees will learn about an autopsy regarding a case of sudden, unexpected death in a hospitalized pregnant young woman, using a complete forensic approach: autopsy, histological and microbiological examinations, and study of medical documentation. In particular, histological findings are the most important elements to find causes of death.

This presentation will impact the forensic science community by showing that myocarditis could be a cause of sudden death, even in cases of a negative test during a hospital admission for therapy of a renal infection in a pregnant woman at 23 weeks, 6 days.

Myocarditis is a histological diagnosis characterized by mixed inflammatory cells within the myocardium. Clinical presentation includes a wide spectrum of non-specific signs and symptoms from the absence of any pathological findings to aspecific signs or symptoms like fever, shortness of breath, chills, cough that can only indicate a pathological status to more specific elements like chest pain and arhythmia. Diagnosis is possible only with an echocardiogram study (low ejection fraction and decreased left ventricular systolic function) and a endomyocardial biopsy. When successfully diagnosed (a very low percentage of cases), myocarditits can be treated with anti-inflammatory drugs (like Non-Steroidal Anti-Inflammatory Drugs); beta-blockers, ACE inhibitors (angiotensin converting enzyme inhibitors), and diuretics to support heart failure; corticosteroid to reduce the inflammatory process that is involving the myocardium. In some cases, a temporary pacemaker is indicated to prevent fatal arhytmia. Prognosis is strictly linked to ventricular function recovery.

A case is reported of a 35-year-old pregnant woman at 23 weeks, 6 days who was admitted for a renal infection two days before death. The woman's clinical history was negative for any chronic diseases but positive for renal diseases cystitis and pyelonephritis (urine analysis of four months and a month before were positive for *Escherichia Coli*), which were treated with antibiotic therapy. Gynecological anamnesis: five years prior she had a pregnancy with cesarean birth and one year later she had a second pregnancy with voluntary termination.

Suffering from pain at the left renal loggia with fever (39.6°C), she went to the hospital. At admission, the objective exam showed a fever (body temperature 38.4°C) with normal cardiovascular parameters (arterial pressure 110/70mmHg, heart frequency 82bpm, rhythmic); at echography, the pelvis and ureter of left kidney were expanded; the urine analysis showed the presence of leucocytes and erythrocytes. There were no abnormalities of pregnancy parameters. Antibiotic therapy (Zinocef (cefuroxime) 1.5g 2/die e.v.) was administered to the woman. The following day the fever was reduced (maximum body temperature 37.4°C) and cardiovascular parameters were regular. The second day after admission, signs and symptoms showed a progressive improvement: apyrexia, reduced level of PCR (from 415mg/l to 212mg/l), normal lymphocyte count. In the afternoon of the same day, the young woman was found unconscious on the floor of the bathroom. The resuscitation was useless and death was confirmed.

External examination of the body was completely negative. Autopsy revealed bilateral subpleural effusions (lung congestion). The heart was normal in size and shape, and the myocardium didn't show abnormal findings except for a thickening of mitral valve. The placenta exam and fetus autopsy didn't reveal pathological findings.

Histological examination showed lymphocytes infiltration of the myocardium, especially in the right ventricle and some outbreaks of necrosis. Other lymphocytes infiltrations were found in the lungs and placenta. The cause of death was attributed to malignant arhytmia by viral myocarditis.

Sudden Death, Myocarditis, Pregnancy

G20 Violence Against Women Victims: Forensic Autopsies Findings in Istanbul

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After attending this presentation, attendees will be familiar with the prevalence of crime against women in Turkey.

This presentation will impact the forensic science community by providing actual data obtained from autopsies performed on victims of violence against women.

Because of a widespread public health problem and a human rights violation, violence against women, and its causes and consequences, carries a particular importance. Violence against women causes women's health deterioration, mutilation, and even death.

Istanbul contains approximately 18% of Turkey's population and is the most important cosmopolitan city in the country. According to statistics from 2006 to 2010 census reports, there are nearly 36 million females living in Turkey. There are 6.5 million females living in Istanbul, and since this cosmopolitan city houses a big portion of Turkeys female population, the result of the study of violent female death can be an indicator for the whole country. This study retrospectively evaluates the results of autopsies performed at

Ministry of Justices Department of Forensic Medicine Mortuary on victims of violence against women for the five year period between 2006 and 2010.

There were 4,165 cases of female deaths out of a total of 20,486 autopsies conducted during this period. The female death cases were categorized as follows: 459 cases of death due to violence against women, 72 cases due to suspicion of murder, and six cases of death not directly related to an act of violence but due to diseases, for total of 537 autopsy cases.

Based on total autopsies (n= 20,486), 2.6% (n=537) of the female deaths were due to violence. Extrapolating these data result in 12.9% of total female deaths being a result of violence against women.

While subjects' age ranged from newborn babies to 90-year-old females, 49.7% of the subjects were women aged 21 – 40. The most frequent violent environment was home, where 51.2% (n = 275) of incidences occurred. Women who suffered violence on the streets were 14.5% (n = 78) of the reported cases. 6.7% of the cases happened at vacant land, while 4.3% of them occurred in buildings. Violence against women in the workplace was 23.3% of the autopsies. The location of one-third of the cases could not be identified.

Although perpetrators of 172 cases were not identified, 20.1% of the violence against women was committed by their spouses. This number increased to 52.3% when we added boyfriends' violence against women. The number of subjects who were killed by family members other than the spouse is 73 (20%). 4.4% of violent individuals were females (n = 16). 10.6% (n = 57) of perpetrators committed suicide after the incident, and only 9.7% (n = 52) turned themselves in to the police station.

76.4% of subjects were found to be dead at the scene, while 15.5% of them died on the way to hospital, and 8.2% died after a period of hospitalization.

According to the autopsy results, death as a result of gunshot wounds (50.1%, n = 269) was identified as the top reason for death, while stab wounds (28.3% (n = 152) took second, and strangulation deaths (8.4%, n = 45) took the third place. Handguns were used in 84% (n = 230) and hunting guns in 13%, n = 35 of deaths. The most common fatal shots were to the head region. Death occurring as a result of stab injuries was determined to be the reason in 172 of the cases, with 42.7% (n = 73) of these deaths due to cuts suffered by victims while defending themselves. Ten cases (2%) were victims of sexual assault, ranging in age between 10 and 83 years old.

Violence against women is gaining more importance in todays world. Although there are several studies showing the extent of violence against women in Turkey, this study is the most comprehensive research on forensic autopsy. Violence against women encompasses a multidisciplinary approach; however, exposing these horrible crimes against women will enable our scientific communities to find a better approach in elimination or reduction of these violent acts.

Women, Violence, Autopsy

G21 Antemortem and Postmortem Colonization Interval by Insects: A Case Report

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After attending this presentation, attendees will understand the importance of the discrimination between antemortem and postmortem insect colonization on a corpse, in order to correctly estimate the time since death.

This presentation will impact the forensic science community by demonstrating that with a complete investigation in a case, it is possible to obtain a complete set of data concerning both the antemortem and postmortem period; moreover, it underlines the efficacy of the diverse knowledge required of a forensic entomologist in understanding the insect colonization of a corpse.

The use of insects and other arthropods in forensic investigations (medicolegal entomology) is considered a gold standard for the estimation of the time since death. However, another important branch of medicolegal entomology is the analyses of dipterous feeding on a host's necrotic or living tissues (myiasis) to determine if the insect colonization can be considered in a forensic context (e.g., neglect of elderly). In some particular cases, a myiasis event can happen within a certain period prior to death, such as the colonization of a bloody lesion, bedsores, or the necrotic tissue of a living person who generally is unable to look after themselves. In such cases, the discrimination between a myiasis colonization and a postmortem colonization based on the insects present on a corpse (species, instar, colonization interval) may provide the time since death (Postmortem Colonization Interval, PMCI) and the time of colonization before death (Antemortem Colonization Interval, AMCI).

In June 2007, the corpse of an 82-year-old woman was found on the floor of her apartment in Turin, located in northwest Italy. The neighbors were alarmed by an odor emanating from her apartment, and when the medicolegal and entomologist arrived, the corpse appeared to be in a fresh stage of decomposition. Rigor mortis was still evident and only blow fly egg clusters were observed on the woman's eyes. In contrast to this, her left foot was largely decomposed and a large number of third instar maggots were present.

No domestic animals that may have fed on the body were found in the apartment, but many diabetic drugs and medication were present. In particular, a medical prescription concerning the treatment of a diabetic sore on the left foot and a medical certification of the woman's senile dementia were found.

Insects present on the corpse were sampled at the scene and during the autopsy with particular attention not to mix the samples from eyes and foot. Part of each sample was immediately fixed and the remainder was reared in a growth chamber until the adults eclosed. A temperature data-logger was placed in the apartment for seven days after the corpse was taken to the mortuary, and the apartment temperature was compared with the temperature data over the same period recorded by the meteorological station closest to the apartment. This procedure allowed extrapolation of the temperature of the scene in the period prior to the corpse being discovered.

The entomological analyses determined the presence of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) eggs on the eyes, and *L. sericata* and *Sarcophaga* sp. (Diptera: Sarcophagidae) third instar on the diabetic wound. These species are typical myiasis insects and they are common in the urban environment of northwest Italy during this period of the year.

The correlation between the entomological data and the temperature information, together with the anatomo-pathological examination, determined that the woman died the day before the corpse was discovered. The PMCI was obtained using the age of the egg cluster on the eyes, the decomposition stage, and the rigor mortis. The diabetic wound on the foot was colonized four to five days before the death. AMCI was obtained using the age of the insects feeding on the lesion. In all likelihood, due to the senile dementia of the woman, she failed to administer her diabetic drugs some days which placed her in a diabetic coma. It would seem that, during the four to five days of coma, insects colonized the wound on her foot, and only after death were her eyes colonized. This case underlines the importance of the diverse knowledge required of a forensic entomologist and understanding insect colonization of a corpse.

Blow Fly, Myiasis, PMI

G22 Child Abuse and Interview of Pediatricians: Incidence in Southern Italy

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After attending this presentation, attendees will have a better understanding of the mechanisms underlying child abuse, risk factors, and the social value of child maltreatment.

This presentation will impact the forensic science community by providing useful data to prevent the maltreatment and psycho-physical consequence of the abuse.

Introduction: Child abuse and neglect are widespread problems found all over the world and have received considerable publicity over the past three decades. Child abuse represents an important cause of infant mortality and it is a sentinel event in a community, reflecting the effectiveness of prevention strategies, social security policy, and primary care for children.[1] UNICEF data (2003) found that in industrialized countries about 3,500 children <15 years die from abuse or neglect annually. Spain, Greece, Italy, and Ireland have the lowest rates of deaths with 0.1-0.2/100,000 children, while the United States and Mexico have the highest rates with 2.2/100,000 children. The child abuse is committed particularly on children younger than four years.[2] Seventy five percent of abuse is not diagnosed because the physicians fail to recognize signs of abuse. Due to this lack of diagnosis the opportunity to intervene early is lost, and many children suffer repetitions of abuse.[3] Currently in the territory of Southern Italy there are not estimates of the size of this problem.

Objective: The goal of this study is to estimate the incidence of the phenomenon of child abuse in Southern Italy, analyzing the population of pediatric medical centers in the geographical areas of Catanzaro and Cosenza (ITA).

Materials and Methods: A questionnaire was given to physicians of seven pediatric centers. The Chair of Legal Medicine collaborated with the Department of Pediatrics Faculty of University "Magna Greacia" of Catanzaro. The sample involved children aged zero to six years, admitted between January 2004 and December 2011. The questionnaire (closed answers) allowed, at each pediatric center, the investigation of case studies, characteristics of victims, social stratification of families, perpetrators of abuse, different types of child maltreatment in relation to age, type of injuries, and psycho-physical consequences on the victims.

Results: The preliminary results are available. Even in relation to data in the scientific literature, the underestimation of the phenomenon of child abuse determined by technical difficulties in diagnosis of psychological abuse and neglect, abuse committed by parents (81, 1%), denial by attackers, unpreparedness of many operators, delays and omission of reports, lack of collaboration between health centers and law enforcement was expected.[4-5]

Conclusions: Because of the increment of child maltreatment and its social consequences, identification and early intervention may help to minimize the probability of future violence and the consequences of child abuse. So, it is essential to extend the competences of the physicians in the identification and documentation of maltreatment in children for prevent child neglect.

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Child Abuse, Pediatricians, Maltreatment

G23 Postmortem Diagnosis of Marfan's Syndrome In Pregnancy: Cause of Death or Incidental Finding?

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After attending this presentation, attendees will have a better understanding of the importance of an adequate Marfans Syndrome (MS) diagnosis to realize an appropriate monitoring of pregnant patients to prevent and control pregnancy-related risk.

This presentation will impact the forensic science community by highlighting prevention pregnancy-related risk in Marfans syndrome.

Introduction: In the vast area of connective tissue diseases are also counted MS, Loeys Dietz Syndrome (LDS), and the Ehlers-Danlos syndrome. These diseases are all characterized by mutations of genes that encode proteins that are the constitutive elements of elastic fibers and that determine elasticity alterations of the involved tissues. Marfan's syndrome (OMIM #154700) is an inherited, autosomal dominant disorder that affects the skeletal, ocular, and cardiovascular systems. The disease displays high penetrance and wide clinical variability both within and between families, therefore a patient may have mild to severe symptoms that may or may not correlate with other affected family members. Although Marfan's syndrome has a range of characteristic morphological features involving the ocular, cardiovascular, and musculoskeletal systems, the phenotype is variable. In addition, mutations have been identified in the gene encoding for fibrillin-1 and also in the transforming growth factor-b receptor 2 (TGF-bR2) gene. In the postmortem diagnosis of these pathologies, it is necessary to detect external anatomical characteristics and peculiar internal signs. Common phenotypic findings are muscle-skeletal and cutaneous alterations. Among the internal signs are found ocular, cardiovascular abnormalities, and pulmonary manifestations. The most common internal signs of vascular origin are structural abnormalities of the aorta. The pregnancy is, for these patients, a high risk event, especially in consideration of the anatomical and structural anomalies that the high weight involves, as well as the intrinsic risks to the pregnancy state.

Case Report: The case of a young woman, pregnant, at the 37 weeks of pregnancy, with a weight of 129kg, operated with a caesarean section, who died two days later for unknown causes was

analyzed. The external inspection of the corpse showed the presence of *facies lunaris*, wide attack of the palate, cutaneous striations, and muscle-skeletal anomalies in correspondence of the right hand. The autopsy stated pituitary hyperplasia, thymic hyperplasia, massive pulmonary embolism, and complete aortic dissection, type A in Stanford classification. Histological examination showed a complete degeneration of the tunica media of vessels affected by the dissection.

Results: In the studied case, the postmortem diagnosis of connective tissue disease, in the absence of aorta rupture, has not been only an accidental finding but a pathological, pre-existing, and concurrent (dissection) cause of the pulmonary thrombo-embolic event, regarded as the cause of death of the young woman.

Conclusions: It is possible to say that pregnancy in obese patients with connective tissue diseases, particularly in patients with aortic ectasia, presents a high risk of mortality and morbidity in relation to the genesis of aortic dissection. For this reason, a correct and adequate MS diagnosis is fundamental, to prevent pregnancy-related risk and realize an adequate monitoring during pregnancy through implementation of a proper diet and timely eco-cardiotographic controls.

Marfan's Syndrome, Pregnancy, Aortic Dissection

G24 Usefulness of CT Scan Before Autopsy in Case of Firearm Homicide

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The goal of this presentation is to explain the advantage of a CT scan for autospy in known cases of gunshot wounds.

This presentation will impact the forensic science community by discussing how CT is currently the preferred radiological investigation before an autopsy in case of homicides by firearm.

The objective of this presentation is to highlight the interest of the use of a scanner in gunshot wounds, but also to show its limits. The use of a scanner before autopsy identifies projectiles or projectile fragments in the body, highlights the bone's and other organ's damages (liver, heart, lungs, brain), defines intracorporeal trajectories, and sometimes establishs a chronology of the different shots. The scanner is useful in order to conduct an autopsy in good conditions and to make adapted samples for microscopic examination. Indeed, the autopsy allows taking some sample tissues (entrance and exit wounds), but also aids in the recovery of projectiles (wads and pieces of bullet jacket).

The scanner also offers the advantage of being interpreted by other experts if necessary, which can sometimes prevent exhumations in cases of disagreement. The interest is also to use scanner pictures at trial that are as representative as photographs of injuries but psychologically less aggressive to the victim's families.

In France, it is necessary for the judges and investigators to know the different shooting distances and define the positions of the victim and the perpetrators during gunfire. To illustrate this, several cases are presented of wound ballistics for which a scan was performed prior to autopsy. The interpretation is immediately made by a radiologist and a pathologist after the acquisition of computer data and a 3D reconstruction is completed, printed, and given to the pathologist before autopsy.

This presentation will be illustrated with many pictures and photographs of entrance wounds, bone lesions (vertebrae, ribs, skull), and exit wounds. The cases presented in this session show an obvious superiority of CT compared to conventional radiology, including digitized radiographs.

Thus, after the scanner, the external examination, and the autopsy, forensic medical examiners or pathologists must be able to define or estimate:

- The initial trajectories (between the muzzle and the victim)
- The firing angles and the direction of the passing projectile
- The skin distances
- The intracorporeal trajectories
- The sequence of shots
- The possible displacements of the victim during the gunfire

The limits of this approach are mainly related to its lower efficiency compared to conventional radiography combined with the autopsy, when a number of projectiles are grouped in a limited area (e.g., skull), or when multiple trajectories intersect with one another.

In conclusion, CT is currently the preferred radiological investigation before an autopsy in case of homicides by firearm. In France, it is gradually replacing conventional radiography, even if the cost is higher (300 € for a body scanner - 200 € for an autopsy). But the limitations of its use are related to the fact that all forensic services do not have console access to a CT scan and radiologist. Also, psychologically, it is difficult to convince doctors, nurses, and patients that the same CT scan can be used all day long for either living or deceased persons

CT Scan, Gunshot Wounds, Autopsy

G25 Cold Case Investigation: The Los Angeles Coroner Experience

Lakshmanan Sathyavagiswaran, MD, and Christopher B. Rogers, MD, OCME, 1104 N Mission Rd, Los Angeles, CA 90033*

After attending this presentation, attendees will understand the importance of archiving evidence and case records, maintaining chain of custody, and following procedures and protocols.

This presentation will impact the forensic science community by bringing closure to families and the community in the forensic investigation of cold cases.

The investigation of various long-unsolved murders in Los Angeles County through the use of modern forensic science techniques, especially recent advances in DNA, will be discussed. Three case examples will be used to discuss the challenges involved, including verification of identification, retrieval of records and photos, authentication of physical and sexual assault evidence, chain of custody documentation, need for following procedures, and maintaining an archive of old procedure manuals including testing techniques/standards. The need to withhold information from family members in the interest of not jeopardizing an investigation is paramount.

Case 1: Involved a decomposed young female found in a drainage culvert in 1985. She was identified by a forensic odontologist after charting of her teeth and comparison with her missing person ante mortem dental X-rays. There was evidence of sharp force trauma in the neck and torso. There was extensive organ tissue loss from decomposition/maggot activity. The use of a tool mark criminalist consultation in case evaluation will be discussed. A suspect was apprehended using DNA techniques and circumstantial evidence. Challenges faced by the medical examiner during trial preparation nearly three decades later included verification of identification. The problem was that the original forensic odontologist was not available due to illness and another dental consultant had to review films, photos, and reports of the original consultant to validate the identification. The medical examiner on case had also charted the teeth independently and this became important in trial preparation, as the removed jaws were not available for review.

Case 2: Involved a 33-year-old female who was strangled and sexually assaulted in 1979 in her Westside apartment. DNA evidence in the CODIS database identified a suspect and linked him with the sexual assault evidence recovered from the victim. Details of the interview process of the suspect, importance of maintenance of chain of custody logs during evidence collections, and detailed knowledge about testing procedures used all became important during trial preparation more than 30 years later.

Case 3: Involved a 15-year-old girl found in a suburb in 1974 with ligature marks on her neck, ankle, and wrists. The case was certified as suicidal hanging. Later, at an inquest, the manner was changed to death at the hands of another. The lack of follow-up by law enforcement and the Coroner-Medical Examiner's Office will be discussed, bringing up the importance of quality assurance in any medicolegal death investigation system.

In 1998, the current Chief Medical Examiner-Coroner reviewed the case because of a zealous detective and prosecutor and reopened the case. Problems in retrieving inquest transcripts after 24 years will be discussed, as they had not been transcribed and there was only one company that had the proper equipment to retrieve the information. Use of DNA techniques in linking the sexual assault evidence taken from the victim to the suspect and ramifications for the community safety in these delays will also be discussed.

Cold Case, Evidence Collection, Archiving

G26 The Use of Several Different Weapons and Ammunition in Order to Make Police and Forensic Investigations Very Difficult: A Specific French Island Behavior That Continues to Grow

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The goal of this presentation is to show forensic and ballistics difficulties and how to specify characteristics when multiple gunshots occur.

This presentation will impact the forensic science community by highlighting an innovative methodology that associates forensic medical examiners and ballisticians in crime cases with multiple different weapons, ammunitions, and shooters.

Currently in France, when mobsters decide to kill someone in a context of conflicts between gangs, the current most popular method is to use multiple people with several types of weapons and ammunitions.

The goal of this presentation is to demonstrate the value and the current need of multidisciplinary work involving forensic and ballisticians during crime scene examination, autopsy, and sample tissues' microscopic analysis.

As an example, this study reviewed cases that happened in the French territory of Corsica, which is known for its passion for weapons and its code of honor. Corsicahas has a unique weapon ownership rate in France. One in 54 inhabitants own handguns which represents a rate ten times higher than ownership in Paris and numbering more than 30,000 weapons legally by only 280,000 inhabitants. Indeed, Corsica has been one of the deadliest regions in Europe since 1995. There were:

- An average of 32 homicides or attempted homicides per year, three times more than the French average (11.7 versus 4.1)
- In 2007, the homicide rate was 7/100,000 against 2.9/100,000 in the rest of France and even 9/100,000 in 2009.

The perpetrators of these attacks have adapted to advances in forensics and ballistics. Now, usually, homicides are carried out by several people using various weapons, several types of ammunition and shooting distances, and different firing angles. Shortly afterward all weapons are found in a burned vehicle.

These methods are intended to delete all traces of DNA (criminals and accomplices) and to prevent weapons from being traced back to other crimes. But these actions are also intended to prevent ballisticians from doing their work (identification of weapons, comparison shots, etc.).

In addition, for forensic pathologists, the multiplicity of lesions and shooting greatly complicates the determination of trajectories, firing angles, skin distances of shots, and, therefore, the position of shooters in relation to the victim.

Different cases were reviewed and studied in a collegial collaboration including forensic pathologists, ballistics experts, investigators, etc. The cases detailed the different types of weapons, various calibers, and gauges that were used that combine handguns (9mm, essentially), Kalashnikov, and shotguns firing buckshot. A summary is provided of the various skin and organic lesions found during the external examination and the autopsy. The projectiles were recovered and examined immediately by the Laboratory of Forensic Science ballisticians.

In a second step, in the laboratory (police department), the clothes were analyzed and firing tests were conducted to determine the skin distances and the firing angles. Then, assumptions are developed about positions of the perpetrators and victim during the crime scene. These assumptions were then validated later by a reconstruction of the crime scene.

This presentation is a practical application of an innovative methodology proposed in France in recent years, which is more and more appreciated by the judges, and which associates medical examiners and ballisticians working in forensic service (police department).

Homicide, Weapons, Multidisciplinary

G27 The Contrecoup Phenomenon: Do Classical Concepts Trump Witness Accounts?

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After attending this presentation, attendees will be able to: (1) describe major biomechanical theories attempting to explain the contrecoup phenomenon; (2) predict likely sites of cerebral contusion in blunt head trauma as a function of the biomechanics of the injury and physical properties of the brain and skull; (3) summarize relevant literature of the reliability of witness accounts; and, (4) stratify likely head trauma scenarios in a case as a function of witness accounts versus knowledge of the contrecoup phenomenon.

This presentation will impact the forensic science community by discussing how the forensic pathologist is often called upon to interpret head trauma as a function of biomechanics, and speculate on relative likelihood of different scenarios, based on the anatomical findings. To the extent that witness accounts may contradict more likely scenarios based on those anatomical findings, it is important for the forensic pathologist to be able to weigh the reliability of both the anatomic findings and that of witness accounts. The two cases presented highlight this controversy, as the biomechanics of the contrecoup phenomenon are discussed in parallel with literature addressing witness account reliability.

It has long been recognized that cerebral contusions tend to occur opposite the point of impact after a fall. Orbitofrontal and inferior temporal contusions, in particular, often result from a fall on the back of the head. This empirical fact has led to complex theories involving the physical properties of the brain and cerebrospinal fluid, angular acceleration, and the elaboration of shearing forces to account for this so-called contrecoup phenomenon, a subject which continues to be debated. Nevertheless, the forensic pathologist is left with the practical implications of such a lesion and is occasionally called upon to offer an opinion about the nature of an injury that may

assist law enforcement officials to piece together crimes and even assign culpability to one individual or another.

Here, two cases of individuals who died as a result of bar fights are presented. In the first case, several witnesses noted that, during the course of an altercation, a 42-year-old man suffered a severe blow to the face, and fell, hitting the back of his head. He immediately lost consciousness, went into cardiorespiratory arrest and was pronounced dead on arrival to an emergency department. At autopsy, he had a severe anterior frontal contusion, bilateral subdural hemorrhage, generalized brain swelling, central herniation, and cerebellar tonsillar herniation.

In the second case, a 30-year-old man was followed from a bar by several individuals who collectively attacked the man with a series of punches and kicks, according to witnesses. At least one of the assailants was also wielding a baton. Importantly, none of the witnesses to the attack noted a fall on the back of the head. The victim arrested at the scene and resuscitation attempts were unsuccessful, and he was pronounced dead on arrival at a nearby emergency department. Despite the reported events, at autopsy, he had a linear skull fracture centered on the right occipital region extending to the skull base, indicating some type of impact to the back of the head. Examination of the brain revealed a large anterior frontal contusion with cerebral swelling, central herniation, and cerebellar tonsillar herniation. The combination of impact injury to the back of the head with a large frontal contusion indicates a contrecoup-type injury. Furthermore, no significant soft tissue injury to the face or forehead was present, making a coup-type injury a less likely cause.

These two contrasting cases raise the issue of the contrecoup phenomena, particularly the reliability of general understanding of the contrecoup phenomenon versus witness accounts. Studies evaluating the accuracy of witness accounts have shown a large variability in the reproducibility and accuracy of recounted events. Details pertaining to physical characteristics and action sequences are commonly evaluated and are more frequently erroneous, despite other aspects of the account remaining reproducible over time. Ultimately, it is concluded that angular acceleration of the brain accompanied by blunt, and essentially, instantaneous impact occurred in both cases. Moreover, a blow to the occipital region of a stationary head is unlikely to account for a large frontal contusion, thereby emphasizing the mechanism of contrecoup injury over witness reports.

Contrecoup, Contusion, Witness

G28 Erroneous Notification of Sexual Abuse in an 8-Year-Old Female, Who Died From Acute Chest Syndrome in Sickle Cell Disease Combined With Deficit of Glucose-6-Phosphate Dehydrogenase

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After attending this presentation, attendees will understand the crucial importance of careful detection and skilled interpretation of anogenital findings in deceased pediatric subjects. Very often, normal postmortem artifacts and physiological variants of genital anatomy, especially in prepuberal children, can be misinterpreted by unskilled examiners and wrongly attributed to sexual abuse, with a poor forensic outcome.

This presentation will impact the forensic science community by improving knowledge of what is normal in anogenital anatomy during the postmortem interval and what is not. Attendees will also focus on the peculiar, different significance that one morphological sign may have on the living's, as opposed to the deceased's, anatomy. The health care practitioner whose sole experience ranges in the antemortem scenario, when operating untrained in a postmortem context, may dramatically confuse common cadaveric anogenital artifacts with traumatic injuries. In the reported case, such an occurrence is described.

An Italian 8-year-old black female of Western-African ancestry, suffering both low levels of glucose-6-phosphate dehydrogenase (G6PDH) and sickle cell disease requiring multiple hospitalizations for micro-occlusive crises, was admitted to the emergency room complaining of bilateral leg pain, fever, and general malaise. Laboratory tests revealed severe anemization (hemoglobin 3.6g/dL) and elevation in hemolysis markers. Clinical course showed acute chest syndrome unresponsive to repeated blood transfusions and intensive care therapeutic efforts, leading to death 48 hours after hospital admission. A few hours after death, during the recomposition of the body, nurses and pediatricians noted abnormal anogenital findings consisting in the absence of hymen and wide dilatation of the anal sphincter. Such findings were suspected as traumatic origin and confirmed by the gynecologist consultant.

Forensic autopsy took place six days after death and showed the following main findings: (1) at the anogenital exam, hypoplasia of the hymen with intact edges, but remarkable dilatation of the anal sphincter with two round-shaped abrasions above the pectinate line; no other anogenital or perineal injury; (2) at histology, rectal abrasions consisted in disepitelization with glandular damage, and microvascular disruption with blood cells and fibrin extravasation; no cellular infiltrate was detected, neither fibrosis or sclerosis in the mucosal and perineal specimens; and, (3) at autopsy, meningeal congestion and cerebral edema; hydrothorax (1200ml), pleural and pericardial adhesions; haemorrhagic edema and diffuse microthrombosis of the lung vessels; erythro- and granulo-blastosis of the lung, heart, and kidney; myocardial hypertrophy and focal myocardiosclerosis; acute tubular necrosis; hepato-splenomegaly with hepatic iron storages.

Autopsy was completed by microbiological analyses of anorectal and cervicovaginal specimens, excluding the occurrence of sexually transmitted infections.

The death of this young patient was related to an acute chest syndrome consequent to a severe sickle cell crisis. Suspected sexual abuse was definitely ruled out by expert examination of the body. In fact, the reported "absent hymen," considering its intact edges and the peculiar morphology, was interpreted as an anatomical variant (semilunar hypoplastic). Anal dilatation was itself explained by childhood anatomy, with underdeveloped glutei showing anal sphincter as "abnormally" evident, and by postmortal dynamic anal alterations. In fact, as reported, sphincter relaxation occurs immediately after death, followed by narrowing during rigidity onset, and by definitive anal relaxation.[1] Furthermore, skilled external examination and histopathology related the two anal "abrasions" to postmortem disepitelization in the site of insertion of a therapeutical suppository, occurred very short time before death.

In conclusion, the interpretation of genital postmortem findings remains an issue of main concern also for the forensic pathologist. In fact, until recently, scarce information existed on the nature and appearance of the anogenital tissues during the postmortem interval. Findings like mucosal disepitelization at different sites of the anogenital tissues have been widely classified as normal postmortem artifacts, but they can be eventually confused with traumatic findings by examiners whose sole prior experience lies in the antemortem forensic context.[2] Moreover, pediatric age makes the interpretation of suspected lesions even more challenging, since relevant interindividual variability in hymen conformation is characteristic of prepuberal young female population.

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Anogenital Exam, Sexual Abuse, Hymen Variability

G29 The Assessment of Refugees for Signs of Torture: An Italian Glimpse

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After attending this presentation, attendees will become acquainted with the medicolegal service for the assessments of refugees in Milan, and in detail, the limits that are usually encountered in the evaluation of signs of torture.

The presentation will impact the forensic science community by providing information in this sensitive and recent field of application of forensic clinical pathology.

Assessment of torture allegations is one of the most recent fields of application of clinical forensic pathology, which is often affected by specific diagnostic limits, especially for what concerns the assessment of scars and the reconstruction of their origin.

In Italy, specific committees actually exist for the recognition of the status of refugee, which only in the last year have begun to include forensic pathologists.

The goal of this presentation is to familiarize attendees with the signs of torture in an initial sample of refugees examined at the Institute of Legal Medicine of Milan in order to show the difficulties which are encountered in establishing a correct coordination between the different types of professionals involved in the specific committees and the pitfalls in the interpretation of lesions and scars.

Twenty-six cases have been assessed thus far between 2008 and 2012. Each alleged victim was interviewed and examined. Every scar and lesion was analyzed, especially those concerning morphology and size, and photographed. In several cases X-rays were taken, for a better understanding of bone calluses.

The final report concerning the consistency between lesions and the given history was performed according to the indications of the Istanbul protocol.

In most cases, the subjects came from African countries and a high number of them went through Libya during the civil riots. Almost all the subjects are male, and, in 56% of cases, they are aged between 20 and 30 years. Only in 32% of cases, the history revealed torture for political reasons, whereas in 28% of cases a history of random military aggression was given. Blunt and thermal lesions were most frequently reported (respectively 49% and 23%). Blunt lesions were most frequently performed by truncheons and wooden sticks (respectively 26% and 24%) and were observed on the limbs and the torso; thermal injuries were performed by torches (45%) and cigarettes (30%) and highly affected the limbs. Sharp force injuries were produced by knives in 70% of cases and were observed most frequently on the trunk. Gunshot lesions, dog bites, and chemical and blast lesions were present.

One of the most relevant results concerns the correlation between scars and the origin of the lesions reported by the refugee. In almost all cases, the analysis led to a general concordance with the presumed history, although other origins could not be excluded. In a few cases, the scars were highly suggestive for the reported history according to the existing literature and concerned specific modalities, such as thermal and sharp force lesions.

This presentation highlights the lack of information in literature concerning the correct assessment of scars and the need for further studies in this relatively recent and sensitive field. Another important point of discussion concerns the need for a correct coordination between the different professional figures involved in such committees in order to produce a useful and clear report of the alleged tortures.

Human Rights, Torture, Refugees

G30 A Bus Crash With School Children in Sierre, Switzerland: Identification of the Victims

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After attending this presentation, attendees will get an overview of the identification process of the 28 Belgian victims who died in a bus crash and learn about difficulties and possible solutions. They will also get an overview of the Disaster Victim Identification (DVI) response in Switzerland.

This presentation will impact the forensic science community by showing how international standards and collaboration aid in the management of a mass disaster.

On March 15, 2012, at 9:15 p.m., a Belgian bus crashed head on against a highway tunnel wall near Sierre, Switzerland. Of the 54 occupants, 46 were children from two Belgium schools on their way home after a ski camp. During the vast rescue operation, the first responders faced many problems because of the confined space in the tunnel. It took time to access and extract the injured and the dead from the bus wreck, as the seats were torn away and stuffed together in the impacted front area of the bus, trapping most of the accident victims. Twenty-eight bodies were retrieved from the bus, including 22 children, four tutors, and two drivers. All bodies were numbered on the site of the accident and transported to a local mortuary with funeral cars. Some local stakeholders contemplated a visual recognition process in order to identify the victims, but the decision for a scientific identification was reached quite rapidly and a request for a Swiss DVI Team operation was issued. A visual confrontation of families and bodies was still expected to be conducted. On the morning of the 26th, the DVI Identification procedure started in the regional mortuary using local means and personnel. These first responders were then reinforced and later relieved by one Belgian and two Swiss DVI teams. All bodies and personal objects were documented (inventoried and photographed), and DNA samples were taken according to a simplified Interpol protocol. Few identifying features were present on most children, and many bodies were heavily traumatized. All bodies underwent a full body CT scan for which they had to be transported to the University Centre of Forensic Medicine of Lausanne (CURML), Switzerland. The autopsies of the drivers and a few dental exams also took place there. The Belgian DVI-Team arrived together with the families of the injured and the dead. The Belgian Team joined the Swiss investigators in order to obtain the antemortem (AM) data. The ability to approach the families in their native language was of paramount importance to obtain high-quality AM data. The presence of the families as well as the political and mass media interventions put enormous pressure on the DVI crews in order to hasten the release of the bodies. The antemortem-postmortem comparisons were performed during the second night of the event and the identifications were all based on several identifiers. A specially designed visual acknowledgement process also gave supportive information. The multidisciplinary validation of the identifications was associating a Belgian DVI-Team member, which provided a mutual endorsement of the results. All 28 victims, as well as one surviving comatose child, were identified according to international DVI procedures within two days after the accident. All bodies were then repatriated in the morning of the third day. This rapidity was only possible because of optimal circumstances (weekday, trained personnel, and rapid access to AM data) and the coordinated action of local police force, forensic pathology, Swiss and Belgian DVI

Teams, and the DNA-Lab, which rendered the comparison results within eight hours after receiving the material. The CT scans of the bodies were not much used in the identification process in this case, because the combination of the other methods sufficed. The CT data did aid in the reconstructive workup, as no autopsies were performed on most victims. The rapidity of the response and identifications, however, also posed problems regarding logistics and psychological aid for the AM and PM teams.

Disaster, International, Identification

G31 The Efficacy of Combining Various Fingerprint Acquisition Techniques to Obtain Examination-Quality Postmortem Fingerprints From Unidentified Human Remains

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After attending this presentation, attendees will understand the problems involved with acquiring fingerprints from Unidentified Human Remains (UHR). The unpredictable condition of UHR is widely known throughout the identification community. This unpredictability leads to the issues involved with acquiring examination-quality fingerprints for identification purposes.

This presentation will impact the forensic science community by exposing the procedures and techniques utilized for daily UHR cases at the NYC OCME and emphasizing the success of their combination and subsequent identification of the deceased. As a result, widespread use of these techniques may help identify the tens of thousands of UHR that are being held within Medical Examiner's/Coroner's (ME/C) offices throughout the United States. Additionally, these decedents will now have a name and can be returned to their families, who will finally have closure as to the whereabouts of their loved one.

Fingerprint acquisition is quite uniform in the realms of the living and crime scene processing where techniques and technology are developed specifically for each purpose. However, the methodology for fingerprinting the deceased is not uniform, and use of techniques must be determined on a case-by-case basis. A major difference in printing the deceased versus the living is the manipulation of the fingerprinting medium (i.e., fingerprint card, ink pad, etc.). When printing the deceased, the fingerprint medium is being manipulated against the decedent as opposed to the living, where the person is being manipulated against the medium. Therefore, in the case of UHR, a combination of traditional and newly developed techniques are required to obtain examination-quality fingerprints. The challenge lies within the unpredictable condition of the UHR that are being printed. Often the UHR are in various stages of decomposition, which can negatively affect the condition of the hands and fingers. Conditions such as rigor mortis, skin slippage, and mummification can all discourage the attempt of obtaining fingerprints from the decedent. Contrary to the notion that the decedent is not printable, ridge detail on the epidermis and the dermis could still allow for acceptable fingerprints despite the aforementioned conditions. As a result, reconditioning techniques should be used followed by attempting fingerprint acquisition techniques.

The techniques described in this presentation are proven to yield examination-quality prints from UHR that range from fresh to significantly decomposed. The wide range of techniques include the use of reconditioning of skin using tissue injection and soaking/rehydration, boiling, manipulation of degloved skin, as well as recording techniques such as ink/card, fingerprint powder/adhesive lifter/acetate sheet, fingerprint powder/casting putty, photography under microscope, and photography using macro settings. While some of these techniques are by no means foreign to forensics, their usage was adapted to serve the cause for fingerprint identification of UHR. Case studies will be presented detailing particularly interesting and/or challenging cases in which a unique combination of these techniques was used that resulted in a positive identification. Statistics will also be provided to further substantiate the efficiency and success of these techniques and the importance of utilizing them throughout the identification community.

Specific resources and supporting data for utilization of various advanced fingerprint acquisition techniques currently available to the forensic community will be provided. It is recommended that ME/C offices and agencies tasked with the identification of UHR become familiar with the various fingerprint techniques which can assist in cleaning and reconditioning remains, leading to the successful recording of examination-quality postmortem fingerprint impressions.

Fingerprint, Unidentified Deceased, Fingerprint Technique

G32 The New York City Office of Chief Medical Examiner Fingerprint Project Strategy — A Model Approach for Standardized and Systematic Postmortem Fingerprint Identification

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After attending this presentation, attendees will understand that, contrary to popular belief, all available fingerprint databases are not linked to one another and, thus many forensic professionals tasked with the identification of Unidentified Human Remains (UHR) are unable to perform complete searches of Postmortem (PM) fingerprint records against all Available Antemortem (AM) fingerprint records. After attending this presentation, attendees will understand the major issues regarding PM fingerprint identification for Medical Examiner/Coroner (ME/C) offices that are not affiliated with Law Enforcement Agencies (LEAs). The submission of PM fingerprint records to various agencies for adequate searching against AM fingerprint records will be discussed, as well as the potential solution developed at the New York City Office of Chief Medical Examiner (OCME).

This presentation will impact the forensic science community by augmenting UHR identification efforts through the proposal of a step-by-step process to utilize current resources available for postmortem fingerprint acquisition, submission, and reporting. As a direct result, utilization of the OCME Fingerprint Project Strategy may aid in the identification of numerous UHR that are currently being held within ME/C offices throughout the United States. In addition, law enforcement agencies will be able to resolve open missing person cases and investigations, as well as close out active warrants. More importantly, the next of kin of these deceased individuals will no longer question the whereabouts of their missing family members and will be able to pursue proper disposition and burial.

Current fingerprint databases utilized by ME/C offices and LEAs exist at federal, state, and local levels, and thus, do not always overlap with the information they contain. In addition, not all ME/C offices and LEA's possess the ability to search every one of these databases—a fact often unfamiliar to many forensic professionals who lack expertise in fingerprint searching and analysis techniques. The OCME Fingerprint Project Strategy was developed to streamline PM fingerprint acquisition, digital conversion, submission, reporting, and case management. Additionally, an electronic fingerprint workflow was developed to integrate with OCME's Unified Victim Identification System (UVIS)/Case Management System (CMS), creating a paperless procedure to minimize manual data entry, redundancy, and reduce chances of human error.

Statistical data from unknown and unverified deceased cases at the OCME will be presented to demonstrate the effectiveness of

utilizing the OCME Fingerprint Project Strategy. As of February 14, 2012, PM fingerprint records were acquired from all available UHR and submitted to multiple fingerprint databases in an attempt to exhaust all efforts for forensic fingerprint identification. Prior to this study, fingerprints from UHR at the OCME were generally only submitted to be searched through a local fingerprint database, yielding minimal or no hits and exhibiting a stagnant and unpredictable turnaround time. As a result, these UHR cases were forwarded to the OCME Forensic Biology laboratory for DNA analysis, a costly and lengthy process that is successful only when AM DNA reference samples are available from a missing person or family members.

A very high success rate was achieved resulting in a multitude of positive identification—many that would have been submitted for DNA analysis. Therefore, the results of this study indicate that to ensure all UHR fingerprint files are being searched thoroughly through multiple databases, PM fingerprint records must be submitted to various local, state, and federal agencies. This would allow maximum exhaustion of all resources to attempt identification of the PM fingerprints. An obvious decline in the number of unidentified and unverified deceased was yielded after the OCME Fingerprint Project Strategy was implemented, confirming a direct correlation and acknowledging the significance of leveraging all available fingerprint databases.

Specific resources currently available will be provided to the forensic community and supporting data regarding the OCME Fingerprint Project Strategy. It is recommended that ME/C offices and agencies tasked with the identification of UHR become familiar with the various fingerprint databases.

Fingerprint, Fingerprint Database, Unidentified Decease

G33 The Contribution of a Digital Fingerprint Protocol to the Medicolegal and Mass Fatality Contexts

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The goal of this presentation is to provide attendees with an example of the benefit of incorporating a digital fingerprinting system into the medicolegal and mass fatality procedures.

The presentation will impact the forensic community by providing data in support of the perceived advantages of the application of digital fingerprint technology in the medicolegal setting. The data presented are extracted from a large medicolegal jurisdiction that acquires the vast majority of its scientific identifications from fingerprint comparison as opposed to other methodologies.

The process of identifying or confirming the identity of the dead is both a core component and a frequent bottleneck in the medicolegal process. This is particularly the case in the mass fatality context, during which the frequent disparity between the time required for scientific identification and the time an average case requires to progress through the disaster morgue can compromise the efficiency of the disaster morgue process. The Harris County Institute of Forensic Sciences (HCIFS) processes approximately 4,000 cases per year, about 800 of which require confirmation of identification. Greater than 90% of these decedents are currently identified using fingerprint comparison, primarily because it is the most efficient and cost-effective option. However, traditional paper and ink fingerprint collection at the IFS required coordination between multiple departments, and considerable hours spent managing the transfer and translation of data. The process regularly takes longer than the remainder of the morgue process from check-in to completion of the autopsy. The HCIFS transitioned to a digital fingerprint process in 2010. The end result has been a significant impact on the time required to identify decedents.

An important distinction is the difference between the turnaround time of the fingerprint identification itself, hereafter referred to as Print Turnaround, and the overall identification (ID) process, hereafter referred to as ID Turnaround (which also includes entry of the fingerprint results into the HCIFS database). Since 2010 was the year that digital fingerprints were made part of the morgue standard operating procedure, the comparative sample was composed of a pre-conversion group represented by cases from 2009 (200 cases) and a post-conversion group represented by cases from 2011 (229 cases). In an analysis of the daily HCIFS caseloads before and after the conversion, it was noted that the conversion to digital fingerprint technology drastically reduced both Print Turnaround and ID Turnaround. This presentation will illustrate the benefits of digital fingerprint technology on the HCIFS daily caseload and on mass fatality incident response.

The median Print Turnaround prior to conversion to digital fingerprints was approximately one-and-a-half hours, with 35% of cases requiring more than two hours, and 18% of cases requiring more than four hours. The median Print Turnaround time following conversion is approximately ten minutes with 94% requiring less than two hours and 89% requiring less than one hour. An additional advantage of the conversion is significant reduction in labor and materials associated with ID. The post-conversion Median ID turnaround for the daily HCIFS caseload is 13 hours as compared to 23 hours pre-conversion. This process involves entry and reconciliation of the fingerprint results for each case into the HCIFS case management system.

The implication of the transition to a digital fingerprint system is that in a mass fatality event with the same demographics as the Harris County population, approximately 90% of the decedents resulting from a Mass Fatality Incident (MFI) can be positively identified via fingerprints before the end of the disaster morgue process, if: (1) prints are taken early during the disaster morgue flow; and, (2) the ID Turnaround is reduced via the disaster morgue operating procedures. This finding has prompted rearrangement of the disaster morgue scheme in the HCIFS Mass Fatality Plan to: (1) place the fingerprint station much earlier in the disaster morgue process; and, (2) to task the Disaster Morgue Data Entry Team with management and rapid entry of fingerprint results. The result is a significant reduction in the separation between Print Turnaround and ID Turnaround, which translates into a substantial boost to the efficiency of the overall disaster morgue process.

Digital Fingerprint, Mass Fatality, Disaster Morgue

G34 Blast Injuries in a Non-Military Setting: Findings and Future Implications

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After attending this presentation, attendees will observe a multi-disciplinary presentation of a non-military blast injury case that will illustrate the pathologic, anthropologic, and neuropathologic sequelae of such injuries. They will also learn of new research directions in the area of blast injuries.

This presentation will impact the forensic science community by describing the findings in a non-military blast injury case, a generally rare occurrence in the non-military setting. This information will directly impact fellows and residents in training, who are not exposed to these types of cases in a routine medical examiner setting. This education will prove useful in mass disaster scenarios.

Blast injuries are a form of trauma most common in military settings with military-grade weapons and ammunition. Because of this, non-military forensic pathologists, medical examiners, forensic anthropologists, and death investigators rarely see blast injuries, and

the associated findings, in a decedent. Investigation of a potential blast injury case may require specific information and/or autopsy procedures that are not gathered in a routine death investigation or autopsy.

The most common non-military setting is industrial accidents. Industries involved with flammable/explosive/incendiary materials can yield situations in which workers can be subjected to the types of forces associated with blast injuries. All workplace fatalities must include an OSHA investigation, as they can be very helpful with providing specific information for that type of industry that would not necessarily be readily available.

In this case, the decedent was working on a chemical tank when the tank ignited. The decedent was not hit by shrapnel from the tank but instead was thrown against a chain link fence. He was transported to the hospital but died shortly after admission. His injuries were consistent with previously described typical blast-type injuries, including internal organ lacerations (without overlying cutaneous injuries), barotrauma, pulmonary trauma and bleeding, atypical skull fractures, and deep white matter hemorrhages in the brain.

The findings in this case bring to light the need to be familiar with blast injuries and the classification schema, which includes primary, secondary, tertiary, and quaternary injuries. This study had the benefit of having an in-house forensic anthropologist and an in-house forensic neuropathologist who were both available for consultation on this case, and were able to provide reports detailing the specifics of this unique type of trauma.

In addition, the case was referred to the working group on a Department of Defense grant for traumatic brain trauma from blast injuries. The working group is hoping to use this case in furthering their research for protecting soldiers from traumatic brain injuries and treatment as well.

In order to properly document and work-up a blast-related injury, it is quintessential to have a firm understanding of the pathologic consequences of blast injuries. By reviewing the case reports and classification schema, forensic pathologists and death investigators can be better equipped to deal with non-military blast related injuries, and possibly help further research to protect military personnel who are exposed to this type of injury on a regular basis.

Blast Injuries, Military, Neuropathology

G35 Death Due to Acute Respiratory Failure After Irritating Gases Exposure: Forensic Diagnosis

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After attending this presentation, attendees will become familiar with the injuries which should suggest the involvement of irritating gases in the death of a victim, in particular, the respiratory tract injuries (pulmonary edema and irritation of the respiratory tract mucosa). Finally, attendees will learn to perform the appropriate samples in such cases.

This presentation will impact the forensic science community by highlighting the risks of death after exposure to irritating gases by law enforcement agents or by an assailant with criminal purpose. With these case reports, the attendees will have help determining if death is consistent with irritating gas exposure, in taking into account the results of histological and toxicological findings, and predisposing factors to fatal outcome.

Irritating or incapacitating gases, in which “tear gases” or “lacrimatory gases” such as CS (ortho-chlorobenzylidenemalonitrile) or CN (chloroacetophenone) aerosols or other aerosol-dispersed chemicals, can be used by policemen to arrest offenders, to control riots, or by offenders to commit assault or robbery. Their physical toxicity is well documented, such as eye, skin, nose, mouth, and respiratory tract irritations. They are considered as non-lethal chemical agents but can be, nevertheless, responsible for acute pulmonary distress syndrome, lethal asphyxia, and death.

Here three cases of death by pulmonary failure after irritating gas exposure are reported: two obese men, 35-years-old and 50-years-old, respectively, were restrained by policemen at home, handcuffed behind their back, faced down on the ground, and died during the interpellation (cases 1 and 2); a third man, 32 years old, was discovered dead in a cave (case 3). Electronic Control Device (ECD) were used in cases 2 and 3.

Autopsy findings were asphyxia signs and conjunctival hyperemia in the three cases, conjunctival petechiae (cases 2 and 3), massive congestion of the mucosa of the trachea and the bronchial tubes in the three cases, overinflated lungs (in cases 1 and 3), massive congestive lungs (case 2), liver of cardiogenic shock (cases 2 and 3), and deep congestion of the organs in the three cases. A hypertrophic heart was discovered in the case 1.

Histological analyses showed hemorrhagic pulmonary edema (cases 1 and 2), partially necrotic (case 1), macrophage in the pulmonary alveoli or bronchi in the three cases, polymorph lung tissue with focal alveolar dilation, alveolar ruptures (case 3), focal necrosis and ulceration of the mucosa of the trachea with hemorrhagic suffusions (case 2).

Toxicological analyses found ortho-chlorobenzylidenemalonitrile in lung tissue and on the clothes of the deceased (case 2), but neither in the blood nor in the tracheal and laryngeal swabs (cases 1 and 2). Phosphoric and sulfuric acids were found on the tracheal and laryngeal swabs (case 3), derived from the ammonium phosphate and sulfate found in a fire extinguisher discovered on the crime scene. Cocaine (0.307µg/ml), cannabis (THC-COOH: 7.7ng/ml), alcohol (0.81g/l), and therapeutic level of doxylamine and valenfaxine were found in blood (case 3).

In cases 1 and 2, it was concluded that death was caused by acute respiratory failure due to irritating tear gas exposure (ortho-chlorobenzylidenemalonitrile) in a closed space (apartment) with possible participation of postural asphyxia (cases 1 and 2) and shocks by ECOs (case 2).

In the third case, it was concluded that death was caused by acute respiratory failure due to the association of a chemical lung irritation by the fire extinguisher gas and an obstructise asphyxia by the fire foam. The involvement of drug intoxication and of the shocks by ECOs cannot be excluded.

Conclusion: In these three cases, acute pulmonary failure can be considered as a multifactorial cause of death. Nevertheless, irritating gas exposure is the main lethal factor in the three cases because of tracheal and pulmonary injuries and the presence of some residue of gas in samples performed. Furthermore, these three cases are illustrative of the predisposing factors to death in case of exposure to irritating gas: dispersed gas in a closed space, obesity, a person under the influence of drugs, prone restraint, and taser gun shocks.

Tear Gases, Irritants, Pulmonary Edema

G36 A Rare Case of Biphasic Malignant Mesothelioma Due to Occupational Asbestosic Exposure

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After attending this presentation, attendees will understand some aspects about the incidence of malignant mesothelioma and the histological classification of this disease, with particular reference to the biphasic mesothelioma histotype with prevalent polymorphic sarcomatoid and desmoplastic aspects.

This presentation will impact the forensic science community by considering the likely gradual increase in judicial autopsies in the next decade for deaths from malignant mesothelioma in subjects with occupational exposure, in order of the expected peak incidence in the coming years. Assuming that the diagnosis of mesothelioma is histological, the purpose of this presentation is to share the experience and opportunity to evaluate rare histological aspects of the case. The malignant pleural mesothelioma is an aggressive tumor with a growing incidence. Clinical studies predicting cases of mesothelioma have estimated that this growth will continue for 10 – 15 years and that in the next 20 years, the number of cases will double in Western Europe, rising from 5,000 deaths in 1998 to 9,000 in 2018.[1]

However, the malignant disease, unlike other asbestos, related diseases, remains a rare tumor, an event considered "sentinel" of exposure to asbestos because of the low incidence in the general population (in Italy 3.42 cases per 100,000 in males and 1.09 cases per 100,000 in females) and of the high causal specificity.[2]

The relationship between asbestos and mesothelioma, first identified by Wagner *et al*. in South Africa, has now been documented all over the world.[3]

The average prevalence of mesothelioma in people with prolonged heavy exposure to asbestos is 2% to 3%, but has reached up to 10% in some series. The latency period is usually 20 years or longer.[4]

Malignant mesothelioma is usually seen in older adults, although well-documented cases in young individuals are on record.[5]

In some instances, a familial clustering has been demonstrated and it was hypothesized a genetic background. In most instances, the initial involvement is in the lower half of a hemithorax but spread to the rest of the pleural cavity is the rule.[6]

The diagnosis of mesothelioma is complicated by a peculiar characteristic that differentiates it from many other cancers, namely the extreme histologic polymorphism.

The differential diagnosis is also very difficult for the fact that the pleural and peritoneal cavities, in addition to mesothelioma, often localize metastases of primary tumors located in distant organs that may also be clinically silent.

It is now widely accepted, and repeated by the most recent treaties, the fact that a diagnosis of mesothelioma can be made only after the histological examination of abundant material (at least 10 grams of tissue according to Cotes and Steel), supplemented by a very accurate survey on the major organs and systems (autopsy or clinical and instrumental evaluation), which allows exclusion with certainty all other possible primary tumor's.

Epithelial mesothelioma is the more frequent (50 – 75%), histological variant, followed by the biphasic form (25 – 30%) and the sarcomatoid one (15 – 20%).[7]

In view of its unusual occurrence, a case of biphasic mesothelioma with prevalent polymorphic sarcomatoid and desmoplastic aspects is reported.

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Biphasic, Mesothelioma, Occupational

G37 First Oviposition Timing of Blow Flies on Human Cadavers

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After attending this presentation, attendees will understand the degree to which the first oviposition timing of blow flies varies depending on temperatures and/or covering and the importance of consideration of this information during estimation of the Postmortem Interval (PMI).

This presentation will impact the forensic science community by providing information that will enhance the prediction accuracy of PMI mathematical models using entomological evidence. As a result, the potential error produced by simply assuming that blow flies deposit eggs shortly after a victim's death is reduced, resulting in a more accurate estimation of PMI.

The goal of this research was to understand the relationship between temperature and the first oviposition timing of blow flies and to test whether covering on a body can delay the first oviposition timing. The hypotheses of the study were first, that fly eggs will be observed on a cadaver earlier in warm weather than in cold weather, and secondly, that covering on a body will delay the first oviposition timing.

It is generally understood that blow flies are the first insects that approach and deposit eggs on a cadaver. Due to the extensive literature on the life cycle of blow flies in various temperature conditions, it is believed that PMI can be calculated by mathematical models developed with great accuracy. However, the first oviposition can be delayed due to a number of factors, and as such, the delayed oviposition still remains a potential source of error in estimating PMI.

This research was conducted at the Anthropology Research Facility at the University of Tennessee, Knoxville. A total of 60 donated individuals were observed each day for the timing of fly egg deposits between April 2011 and March 2012. Approximately half (n = 36) of the individuals were covered in black plastic sheeting, while the rest were uncovered and unclothed.

For this study it was found that the first oviposition timing was largely dependent on temperature. In the month of July, when the monthly temperature was 28°C, which was the highest of the year, fly eggs were observed within 24 hours after placement of bodies at the facility. Also, from May to September, when monthly temperatures were above 20°C, it took three days or less for the first oviposition to occur. Conversely, in January, when the monthly temperature was 6°C, which was the lowest of the year, fly eggs appeared about 19 days after placement on average.

More specifically, the first oviposition timing was found to have varied more in cold weather than in warm weather. From May to September, it took between one and three days for the first oviposition to occur. However, in January, fly eggs were observed between 10-28 days after placement. Even though average monthly temperatures of October and March were equal (16°C), the first oviposition timing of October was faster than that of March. This difference can be attributed to the population density of currently existing flies. That is,

in October, at the end of the summer season, it is likely that there still remain a large number of active flies as well as pregnant flies; however, in March, at the end of the winter season, it is likely that there are few active flies over the landscape and even less numbers of pregnant flies. Because there are not many existing flies at this time that are prepared to oviposit, it is likely to take some time for flies to emerge and mate.

Finally, the timing of the first oviposition was only marginally affected by covering. While black plastic sheeting delayed the first oviposition timing during summer, the delay was one day at most. Unfortunately, this study cannot present the effect of black plastic sheeting during winter because all the cadavers placed between December 2011 and March 2012 were covered in black plastic sheeting.

In conclusion, forensic anthropologists and entomologists must be aware of the varied effect of temperature and covering on the first oviposition timing of blow flies. This detailed information should be taken into account when PMI is calculated using blow fly evidence.

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Oviposition Timing, Blow Fly, Postmortem Interval

G38 Evaluation of Head Trauma With Antemortem Radiology and Postmortem Pathological Data

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The goal of this presentation is to show how antemortem imaging data including conventional radiography and Computed Tomography (CT) were compared to the postmortem pathological data that served as the gold standard with regard to common forensic neurotrauma findings such as skull fractures, soft tissue lesions of the scalp, various forms of intracranial hemorrhage, contusion, axonal injury, signs of increased brain pressure or herniation, edema and ischemia. In this study, the purpose is to establish if any difference between radiological and autopsy data occurred in the short period of time after trauma and if conventional radiography and CT is beneficial for forensic head and brain examination.

This presentation will impact the forensic science community by showing how cases underline the importance of a detailed investigation and a thorough evaluation of all circumstantial, clinical, radiological, and autopsy data in the reconstruction of forensic fatalities to identify any possible legal responsibilities of anyone.

Introduction: Head trauma is one of the leading causes of traumatic deaths. The radiological reconstruction of trauma before the autopsy will guide exploring the circumstances of many forensic deaths including occupational, accidental, and medical malpractice cases, especially in the case of hospitalization for a long period of time. The increasing applications of forensic radiology and the wide use of conventional radiography and CT in clinical practice also demonstrate the potential of these technologies as tools for verifying the correspondence between an unidentified body and an identity suspect. CT is the imaging modality of choice for 2D and 3D documentation and analysis of postmortem findings including fracture systems, pathologic gas collections (e.g., air embolism, subcutaneous emphysema after trauma, hyperbaric trauma, decomposition effects), and gross tissue injury. Various post-processing techniques can provide strong forensic evidence for use in legal proceedings. Besides, Magnetic Resonance (MR) imaging has also had a greater impact in demonstrating soft-tissue injury, organ trauma, and nontraumatic conditions.

Methods: The antemortem imaging data, including conventional radiography and CT, were compared to the postmortem pathological data that served as the gold standard with regard to common forensic neurotrauma findings such as skull fractures, soft tissue lesions of the scalp, various forms of intracranial hemorrhage, contusion, axonal injury, signs of increased brain pressure or herniation, edema, and ischemia

Findings: Reports of both postmortem pathological and the antemortem radiological examination of brain and skull in 31 head trauma cases were evaluated. Age range was between 1 to 90. Gender included 25 male and 6 female cases. Any distinct difference between the antemortem imaging and the autopsy data was not found in the case of skull fracture and various forms of intracranial hemorrhage. Axonal injury and contusions were detected more sensitively with the autopsy than with the imaging techniques. During the time the autopsy and radiology reports were performed other brain lesion findings were discussed.

Conclusion: The cases underline the importance of a detailed investigation and of a thorough evaluation of all circumstantial, clinical, radiological, and autopsy data in the reconstruction of forensic fatalities to identify any possible legal responsibilities of anyone. The documentation and analysis of antemortem findings with CT and MR imaging is investigator independent, objective, and noninvasive and will lead to qualitative improvements in forensic pathologic investigation. In addition, as with other morphological methods for identification, comparisons between antemortem and postmortem data require standardization and statistical analysis, especially concerning the admission in court of evidence obtained by anthropological and radiological methods. In consideration of these facts, radiological techniques have the power to play an important role in the forensic pathological examination.

Head trauma, Radiology, Autopsy

G39 An ELISA Examination of Pro-Inflammatory Proteins in Human Blood Samples: A Potential Means for Investigating Diabetes in Postmortem Human Remains

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After attending this presentation, attendees will become familiar with cytokines and their relationship to the metabolic disorder diabetes mellitus.

This presentation will impact the forensic science community by demonstrating how blood cytokines may be successfully analyzed in human remains, and how results may be used to suggest diabetic condition. This is the first time that cytokine data has been applied to postmortem material to provide additional information that may advance human identification in forensic contexts.

The number of individuals suffering from diabetes has reached epidemic proportions globally and within the United States. In 2007, diabetes was cited as the seventh leading cause of death in the United States and was a contributing factor in another 160,000 deaths.[1] Forensic anthropologists must be prepared to recognize diabetes in skeletal remains as they would any other well-documented pathology. The purpose of this research is to investigate diabetes in postmortem material employing blood serum protein analyses.

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia and disturbances in carbohydrate, fat, and protein metabolism, resulting from defects in insulin production and/or function.[2] Insulin regulation is crucial because this hormone is primarily responsible for transporting blood glucose into cells, providing a vital energy source. The present study will selectively focus on Type 2 diabetes, as it is most prevalent and the only form of diabetes documented in the William M. Bass Skeletal Collection.

The predominant theory explaining pathophysiology of diabetes involves activation of the immune system in response to metabolic stress, caused by over-nutrition. Normal physiological response to exogenous stress triggers inflammation of the affected tissue. When stress is acute, inflammation has positive effect, resulting in cell repair and tissue regeneration; however, when the condition is chronic, like obesity and insulin resistance, inflammation may become deleterious and maladaptive.[3] Researchers have identified that patients suffering from Type 2 diabetes demonstrate inflammation in tissues related to adiposity and glucose handling.[4] Studies have uncovered autoimmune inflammation in the insulin-producing pancreatic β-cells of diabetics. Cytokines are cell-signaling proteins that serve as primary mediators of immune responses. Many cytokines are secreted by adipocytes, demonstrating a link between obesity and insulin resistance. Pancreatic inflammation has been termed "insulitis" and may be is characterized by elevated levels of leukocytes and cytokines in the blood stream.[5] Biomarkers of inflammation, like cytokines, can be quantified and may be used as proxy variables to track the progression of insulin resistance.

The William M. Bass Skeletal Collection provides a unique opportunity to investigate diabetes in an extensively documented modern population. Blood sample collection was initiated in 2008 by the UT Forensic Anthropology Center for donated human remains. For this research, four preliminary samples were selected: two known diabetics and two non-diabetics who were pair-matched based on demographic characters (age, sex, ancestry, and body mass index). Diabetic status must be assumed from self-reported medical history.

Blood samples were extracted from the bloodcard paper matrix using a repo-buffer solution. A Bradford Quantification Assay was used to test and verify sufficient amounts of proteins present in each sample. Samples were then subjected to an ELISA multiplex. ELISA analyses determine the presence and concentration of an unknown biomarker. The multiplex investigated 26 pro-inflammatory cytokines. Elevated values were determined in eleven cytokines, six of which warranted statistical testing. Significant difference between the diabetic versus non-diabetic samples was assessed with univariate ANOVAs with a single fixed factor. Assumptions of normality and variance were tested and proven with a Shapiro-Wilks and Levene's test (respectively). To avoid Type 1 error, a Bonferroni Correction was applied (modified α=0.008). After adjusting the alpha, one cytokine (IL-8) significantly differentiated between sample groups (α=0.001); however, another cytokine (MCP-1), demonstrated a tendency toward significance prior to Bonferroni adjustment (α=0.019) which may be recognized given a larger sample size. Both of these cytokines show high correlation with insulin resistance, and are reported to interact with bone cells

Results from this preliminary study indicate that cytokines may be successfully tested in postmortem samples. Data may be used to suggest diabetic status in unknown remains.

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Postmortem Pathology, Diabetes, ELISA Analysis

G40 The Role of *Lepas Sp. Crustacea Cirripedia Pedunculata* in the Evaluation of Postmortem Interval in Aquatic Environments

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After attending this presentation, attendees will understand what investigative role water fauna plays in the evaluation of PMI and PMSI in cases where the discovery of corpses is in aquatic environments.

This presentation will impact the forensic science community by demonstrating how product and entomological surveys could give clues on the origin and permanence of an unidentified corpse.

Introduction: The interval between death and the discovery of a body is known as postmortem interval. Backdating the time of death is useful for resolving legal issues when a corpse is recovered. It is particularly useful to: (1) know when the crime was committed; (2) identify the victim; (3) exclude some suspects in the murder case; (4) direct the investigations of law enforcement; and, (5) verify the statements of a suspect. Among the methods used to calculate the PMI include: analysis of consecutive abiotic phenomena (algor mortis, rigor mortis, livor mortis), analysis of ocular changes, analysis of cadaveric fauna (insect larvae, insects, fish, shellfish), and analysis degree of decomposition.[1-4] In particular, the permanence of a water body causes alterations of both phenomena cadaveric (putrefaction) finds in the exterior and interior of the body. The permanence of the body in water can result in the appearance of cadaverous transformative special phenomena, such as the saponification, with consequent formation of adipocere, and maceration. Of particular interest is the relief of foreign materials and lesions produced in the aquatic environment. These lesions can be produced by the impact of body against rocks and cliffs or into water fauna (vertebrates and invertebrates) and it can cause different types of lesions or sometimes some problems in the distinction of antemortem and postmortem lesions. The wildlife that tends to colonize the corpse in the water allows the evaluation of Postmortem Submergence Interval (PMSI), due to the different timing of colonization of the body by the aquatic fauna and different species of algae found.[5]

The goal of this study was to estimate the time of growth of some types of shellfish to determine the postmortem interval and PMSI. This investigation, in case of naval or air mass disasters, also allows one to determine the possible origin of the body relative to the place where the corpse was found.

Case report: A male subject found on a beach in Central Tyrrhenian of the Southern Italy was analyzed. The external examination showed a corpse in a state of saponification with some areas of maceration from a long stay in the water. For this reason, the external somatic features couldn't be recognized. A survey of clothes was carried out and showed the presence of specific clothing imported from the United States and Italy. Also on the trousers of the victim were crustaceans that were analyzed. In particular, the species found were categorized as belonging to the family of *Lepas sp. (Crustacea: Cirripedia pedunculata)*. The autopsy was completed at the Unidentified Corpse Board (RiSc) for the Ministry of Justice in Italy, then sent to a competent authority. Following the compilation of the board we were contacted by the Police Station of Giglio Island for a possible correspondence with a missing passenger on the Costa Concordia, whose shipwreck occurred on the Ligurian coast January 13, 2012.

The results of autopsy showed the saponification resulted from long permanence in water (three months). An entomological survey was carried out on the time of growth of these crustaceans and the natural habitat of the same was studied. The data obtained was

compared and showed an elapsed time from death to the discovery of approximately three months. This time interval was compatible with the Costa Concordia's sinking. For many reasons, the genetic investigation was performed to confirm or reject the hypothesis.

From a scientific point of view, this study is important because it emphasizes the role of marine life in the evaluation of PMI and PMSI, especially in cases of discovery of unidentified bodies in the aquatic environment. This resolves, at times, problems related to the origin of the corpse from the place where it was found or any information on the country of origin where the accident occurred.

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PMI, Aquatic Environments, Forensic Pathology

G41 The Hidden Side of Sudden Cardiac Death: Forensic Experimental Protocol and Its Applications

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After attending this presentation, attendees will understand the role of gene mutational analysis in sudden cardiac death.

This presentation will impact the forensic science community by discussing the importance of a rigorous genetic testing protocol in sudden cardiac death diagnosis when autopsy examination is not performed.

Introduction: Sudden Cardiac Death (SCD) is death from cardiac causes within one hour of the onset of symptoms. The sudden death of young people is a devastating event for both the family and community. Over the last decade, significant advances have been made in understanding both the clinical and genetic basis of sudden cardiac death. Most commonly, sudden cardiac death can be the first presentation of an underlying heart problem, leaving the family at a loss as to why an otherwise healthy young person has died.

At external examination on a victim of SCD, the features of hypostasis are the most important gross findings. On examination, hypostasis in these cases show, especially, a typical dark red/blue color on the face, neck, and anterior thoracic wall. There is a deep cyanosis on lips and nail beds. The diagnosis is suggested by the absence of external gross injuries. Genetic factors are important factors to the risk of SCD. Coronary artery disease is the major determinant of SCD, and its predisposing genetic role is complex. The other main causes of sudden cardiac death are some hereditary non-structural diseases such as Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), long QT, and the Brugada Syndrome, as well as structural diseases such as Hypertrophic Cardiomyopathy(HCM) and Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) which cause a significant number of cases of sudden cardiac deaths in the young. Many times for economic reasons, in the cases of SCD judicial authorities do not require an autopsy examination performed by a forensic pathologist. As an unfortunate result, forensic investigation of SCD often involves only the external examination of the victim's body. Especially in these cases, genetic testing may be useful because many mutations in several genes are implicated in arrhythmic syndromes including SCN5A, KCNQ1, KCNH2, RyR2, and genes causing HCM, specifically MYH7 and MYBPC3. In these cases of SCD when autopsy examination is not performed, an important progress in diagnosis depends on the use of a rigorous protocol in order to analyze biological samples of saliva of the victim according to the informed consent of the victim's relatives.

Case Reports: Twenty cases of sudden cardiac death were analyzed. In these cases, autopsy examination was not required. For 18 cases, forensic pathologists made external examination and for two cases autopsy was carried out. In these last cases, the cause of death was thromboembolic death ARVC. In the others, the cause of death remained unknown.

The goal of this study is understanding the importance of investigation protocol in order to investigate gene mutational analysis in both structural and non-structural genetic heart disease including SCN5A, KCNQ1, KCNH2, RyR2, MYH7, and MYBPC3. If the victim's test is positive, this information is important for relatives who might themselves be at risk of carrying the disease-causing mutation.

Conclusions: This protocol has to be applied in all cases where forensic pathologists must identify the cause of death without autopsy examination. The main goals of this protocol is to evaluate exact incidences of cardiac causes of sudden death, counseling for victims' relatives, and potential therapy for genetic mutations carried.

Sudden Death, Autopsy, Genetic Mutation

G42 The Role of Forensic Investigations in Child Abuse: Two Case Reports and a Review of Literature

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After attending this presentation, attendees will be able to describe the impact of the forensic science in cases of rape in children.

This presentation will impact the forensic science community by providing information to allow a method that recognizes signs of sexual abuse of minors.

Introduction: This research explores sexual violence, which is a major problem for healthcare. One in six people are victims of rape or attempted rape. A total of 20% of women and 10% of men say they had experienced sexual abuse during childhood. There are 40 million children under age 15 who suffer abuse and neglect. Disabled children are at a level of risk for physical and sexual abuse more than double that of healthy children. The 1.5% of total violence is the incestuous relationship within the family. The proportion of other relatives who commit sexual violence is 4%. All forensic investigations of the victims of rape left particular signs: hyperthermia, bruising, laceration of the mucous membranes of the genitals or perigenital regions, body injuries, marks of constriction, junction lesions by excitement or torture, and the presence of biological traces. A careful review of the literature revealed that the major problem for forensic investigations is detecting signs of violence on the victims. The research demonstrates that many conditions have been mistaken for signs of abuse. The etiological diagnosis based on physical examination is often difficult because the physical signs are

not always present and, if they are present, can be ambiguous; however, for a forensic pathologist it is essential to differentiate accidental injuries and inflicted injuries.

Case Reports: Two cases of incest are reported. One act of violence was perpetrated on a young girl (12-years-old) by the father and an uncle. In this case, the victim filed a complaint six months after the incident, but through careful examination by the coroner, the presence of a hymen was found without evident marks of violence. In this case, the hymen of the young girl revealed elastic characteristics. For this reason, the forensic pathologist could confirm violence occurred and was also confirmed through the testimony of the child. In the second case, a young boy (12-years-old) affected by mental retardation was analyzed. The violence was perpetrated on the child by his uncle. The forensic examination established the presence of possible signs of violence. Also, in this second case, there was confirmation of violence from the child's testimony. These unusual cases were solved by forensic ivestigation. Through forensic investigations the accused were sentenced. The results of forensic examinations were compared with psychological examinations on the victims. In both cases it confirmed the forensic physician results.

Conclusions: For victims of rape, the forensic medical examination is the first step toward solving the crime. In cases of sexual violence, the role of forensic pathologist, through expertise and training, is crucial for the analysis and production of visual evidence. This presentation shows that the uncertain cases were solved with the help of forensic documentation and sciences evidence. Therefore, in order to be more efficient, this study proposes the creation of a multidisciplinary center for anti-violence, implementing a specialized system. In this center, the intervention of a specialist team will allow for correct identification and interpretation of exterior signs and marks on the victims. This team will consist of the forensic pathologist, gynecologists, psychiatrist, pediatrician, forensic geneticist, and other operators. This study indicates that this system is adjusted to various types of forensic cases, and many will benefit.

Child Abuse, Forensic Pathologist, Rape

G43 Technical Data of Intimate Partner Homicide-Suicide in Turkish Mass Media Between 2008-2011 Through Autopsy Reports

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After attending this presentation, attendees will have an idea of technical details of intimate partner homicide-suicide autopsies.

This presentation will impact the forensic science community by discussing the procedures of autopsy reporting and autopsy findings in intimate partner homicide-suicide cases through optimal standards.

The term "Homicide-Suicide" (HS) can be defined as "the committing of suicide by the same individual after his/her commitment of a homicide." HS is most frequently seen among intimate partners and, within this scope, is an important part of the sexual violence in society. Besides, violent features of these incidences mostly find a place in mass media. This study is intended to elicit scientifically the technical data, or autopsy findings, of these mass media cases which have been constituting a good "image" of the general "picture" all around the country about Intimate Partner Homicide-Suicide (IPHS).

A media monitoring agency has been used with specific keywords to detect all the detectable incidences of IPHS which had found place in mass media between 2008 and 2011 in Turkey. Then, the cases were searched in the Turkish judicial system through the autopsy report archives of forensic medicine units all around Turkey, which are organized in the structure of the Council of Forensic Medicine, a unique official forensic medical expertize institution, as a whole. No matter how long the optimal interval between homicide and suicide is defined, in this study all cases for which "homicide-suicide" term between intimate partners has been used in autopsy reports between January 1, 2008, and December 31, 2011, have been accepted as IPHS. Judicial records were evaluated in order to have the autopsy data of the victims. The data collected were analyzed through descriptive statistics and chi-square test with predictive analytics software.

Among 122 IPHS cases, only the autopsy reports of 96 cases could be found. For the remaining 26 cases, the reason for the lack of an autopsy report was either the victim's burial without autopsy through only a death examination report or the autopsy's being performed by another physician (most probably not a forensic medicine specialist) working out of the Council of Forensic Medicine.

According to obtained data, among 96 victims with autopsy reports, only three victims were found to be males. The most frequent method in homicides was determined to be firearm injury (69.7% (n:85) pistol, 18.9% (n:23) shotgun), as the total 89.6% (n:86) of the victims had been killed with a firearm. Lethal lesions were most frequent, as 32.6% (n:28), on the head as a single lesion. There were, in all, 217 injuries resulting from gunshots on 86 victims killed with a firearm, while there were at most 11 injuries per victim with a mean injury number of 2.26 for each victim. When checked according to the features of these injuries, 19.8% (n:17) of the injuries were from contact shots, 10.5% (n:9) were from near contact shots, 20.1% (n:18) were from intermediate range shots, 19.8% (n:17) were from distant shots. Toxicological tests were performed for 75% (n:72) of the 96 victims. Among 72 victims, ethanol was found in 12.5% (n:9).

Among perpetrators' suicides, again the most frequent method of suicides was firearm injury (69.8 % (n:84) pistol, 20.5% (n:25) shotgun) as total 89.6% (n:86) of the perpetrators had committed suicide with a firearm. Lethal wounds were most frequent, as 76% (n:73), on the head as a single wound (with a pistol in 80.8% [n:59], with shotgun in 17.8% (n:13), with falling from a height in 1.4% (n:1). The suicide of the perpetrator was more frequently and statistically significant in the first hour after the homicide, and the method was more frequently and statistically significant with the same homicide method. Toxicological tests were performed for 71.9% (n:69) of the 96 perpetrators; among 69 perpetrators, ethanol was found in 12.5% (n:9) and insecticide was found only in one perpetrator explaining the origin.

Besides the discussion of these technical details above about the nature of injury, the ratio of autopsy in IPHS cases as 96/122 indicates some legislation problems in Turkey in letting other physicians perform autopsies or allowing burial without a forensic medicine specialist's consent, but instead with the assignment coming just from the prosecutor. Quality standards like 99/3 Autopsy Harmonization Standards across Europe or any ISO 17020 execution can help to convert the legislation according to scientific standards of authorization in autopsy performance.

Homicide-Suicide, Intimate Partner, Autopsy

G44 Oblique Lighting Applications in Forensic Sciences

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The goal of this presentation is to demonstrate the effective use of oblique light to enhance surface texture and illuminate subtle features in a forensic setting.

After this presentation, attendees will have a better understanding of when and how to use the oblique lighting technique during a forensic examination.

Photographic documentation of autopsy findings is of particular importance, especially in cases which could result in judicial

proceedings. When in court, expert witnesses use these visuals to present what was initially observed during the examination; therefore, documenting accurate images are vital for this process. Typical photography technique used during autopsy examinations for documentation involves perpendicular direct light on the region of interest. Oblique lighting is commonly used to reduce glare that may obscure the subject matter. This presentation discusses the use of oblique lighting to highlight subtle features and traumatic injuries so that detailed visual documentation is available. This technique was used in three different types of examinations in a medical examiner setting: pathology, neuropathology, and anthropology.

The study was conducted during a three-month Forensic Photography Internship Program at the Harris County Institute of Forensic Science (HCIFS), Houston, TX. Photographs of 15 cases were taken as part of the autopsy using the standard operating procedures defined by the Forensic Imaging Division. Photographs were taken using a digital SLR camera fitted with a 35-70mm lens as well as a 105mm macro lens. The camera also included a digital SLR off-camera Through the Lens (TTL) flash and a digital SLR TTL off-camera flash cord. The cases photographed required one or multiple types of examinations including pathology, neuropathology, and anthropology; therefore, the subject matter included both soft and hard tissue. Photographs were taken at the request of the forensic pathologist as needed during the autopsy for each case. Neuropathology photographs were taken after the brain and eye samples were formalin-fixed and dissected for examination. Anthropology photographs included cases recently exhumed as part of the HCIFS Unidentified Decedent Review. Photographs were taken prior to processing the exhumed remains as well as after the remains were processed for anthropology analyses. All the cases photographed for this study used direct perpendicular light and oblique lighting to demonstrate the efficacy of oblique light when surface texture details are required.

Results show that when oblique lighting is applied the amount of detail being documented is increased. In pathology examinations, soft tissue impressions were highlighted when oblique light was used. The lighting was best used in cases of motor-vehicle accidents to emphasize the presence or absence of seatbelt injuries; in cases of gunshot wounds where the presence of Gunshot Residue (GSR) needed to be recorded; and in cases of hanging/strangulation where the details of the ligature furrow needed enhancing. To document neuropathology examinations, oblique lighting was used to illuminate the eye to visualize retinal hemorrhages as well as to reduce the glare caused by vitreous fluid. These types of hemorrhages are particularly important in suspected child abuse cases. With photographs of the brain, oblique light reduced the glare typically encountered with direct perpendicular light. In anthropology examinations, oblique light successfully captured billowing on the surfaces of the pubic symphyses as well as epiphyses from the long bones of young individuals. These features are particularly important in estimating age. Furthermore, oblique lighting was best suited to document cases of subtle perimortem injury, in particular sharp force trauma, when present on bone.

This study demonstrates the usefulness of oblique lighting for photographic documentation of subtle surfaces details acquired during examinations in a medicolegal setting. These details play a vital role in assessing cause and manner of death and are especially important in cases where this information will be presented in court.

Forensic Photography, Oblique Lighting, Technique

G45 Death Due to Renal Toxicity Following Bath Salts Abuse

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The goal of this presentation is to illustrate a case of death associated with the use of Psychoactive Bath Salts (PABS) and the associated renal findings.

This presentation will impact the forensic science community by illustrating the unusual renal findings in a death associated with PABS and a novel theory.

The abuse of PABS has become increasingly common in the United States since being introduced in the early 2000s. Cases of overdose are increasing in presentation and significant morbidity and mortality have been reported.

PABS belong to a family of drugs known as synthetic cathinones. Cathinone is a naturally occurring amphetamine analogue found in the *Catha edulis* plant. The most commonly isolated synthetic cathinone found in PABS appears to be Methylenedioxypyrovalerone (MDPV). In one study analyzing patient blood, urine, and PABS samples, no other synthetic cathinones other than MDPV were detected. Testing for MDPV poses an issue in forensic medicine as MDPV is not commonly screened for in routine toxicological testing and may cause a false-positive result for Phencyclidine (PCP).

The effects of PABS are similar to those attributed to other amphetamines and central nervous system stimulants by inhibiting dopamine and/or norepinephrine reuptake. Rhabdomyolysis, hepatic and renal failure, and cardiac and neurologic toxicity have been reported.

Although illegal in the United States, PABS are reportedly accessible via the Internet and are commonly produced overseas, often in China. Currently, the short- and long-term physiologic effects of PABS and their etiology are not fully understood.

Materials and Methods: The case involved a 42-year-old Caucasian male with a history of alcohol abuse, Hepatitis C, and liver disease. He was found unresponsive by family members at his residence where he had reportedly been ingesting PABS.

Initial toxicology screening performed at the treating hospital was positive for PCP and opiates and negative for alcohol. He ultimately developed multisystem organ failure and sepsis after a prolonged stay in the intensive care unit. Death ensued thirteen days after initial presentation. Treating physicians could not rule out a possible overdose.

Results: External examination revealed mild anasarca, moderate pulmonary congestion and edema, jaundice, and one liter of ascites fluid. Microscopic examination of the liver revealed portal lymphocytosis and stage III bridging fibrosis compatible with the clinical history of Hepatitis C virus. Sections of the kidneys demonstrate mildly dilated tubules containing round to ovoid, lightly eosinophilic, green crystals with concentric peripheral lamellations and central radiations, both singly and in small aggregates. Literature review revealed that these crystals are compatible with melamine/cyanuric acid crystals.

Conclusions: Considering these findings in the context of reported PABS abuse and what little is known about PABS manufacturing, it is possible that melamine toxicity contributed to the renal failure in this case. Melamine is an organic compound used in the production of plastics, dyes, fertilizers and various textiles. In the late 2000s, melamine was found to be present in several products produced overseas including pet food, livestock feed, and infant milk, where it was used as filler to falsely increase the protein content.

Both the acute and chronic toxicity of melamine has been well documented. In one study, sheep fed a single 100g dose of melamine all died of renal failure within 11 days.

In this case, melamine may have served as an unintentional contaminant of PABS manufacturing or as filler to falsely increase the

amount of the crystalline drug. Future plans for research are to analyze various PABS with both Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectroscopy (GC/MS) for the presence of melamine.

Additional pre- and postmortem investigation is required to further establish an etiology of multisystem organ toxicity in PABS abuse.

Bath Salts, Melamine, Renal Failure

G46 The Ability to Shoot Back: Review of Multiple Suicidal Gunshot Wounds to the Chest

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The goal of this presentation is to illustrate three cases of multiple gunshot wound suicides involving seemingly incapacitating injuries on the initial attempt with a firearm with the ability to follow-up with a second gunshot.

This presentation will impact the forensic science community by illustrating the ability to survive extensive and devastating damage caused from an initial gunshot wound, and the potential for physical activity to inflict additional gunshots with a firearm.

Multiple gunshot wound suicides are encountered infrequently but are well documented in forensic pathology. The literature covers a range of unusual circumstances and settings that have involved common target sites and the use of multiple firearms. The cases are often challenging to law enforcement personnel and to the pathologist.

Suicides involving firearms are generally lethal and certain vital regions of the body are targeted for rapid incapacitation. These areas are physiologically necessary to maintain central nervous system function. The most common sites are gunshots to the head causing direct disruption of cerebral tissue, and gunshots to the chest causing cerebral hypoperfusion and hypoxia from massive blood loss from the heart and major blood vessels. The possibility of physical activity after sustaining massive damage is crucial in the reconstruction of shootings involving more than one gunshot wound.

Materials and Methods: Three cases of multiple suicidal gunshot wounds to the chest are presented. All three cases involve Caucasian males ranging in age from 40 to 87 years with two self inflected gunshot wounds to the chest region. The investigative report and scene photographs are reviewed for type and caliber of firearm and the autopsy report and photographs are reviewed for bullet injury profiles.

Results: The first case (87-year-old) revealed two contact penetrating gunshot wounds to the left chest with one bullet causing injury of the left lower lobe of lung and the second bullet perforating the thoracic aorta and heart. Associated injuries included a left-sided 400ml hemothorax, 200ml of hemopericardium and periaortic hematoma. The firearm was a .38 special revolver.

The second case (40-year-old) revealed one contact penetrating gunshot wound to the right chest with bullet injury to the middle and lower lobes of the right lung and the liver and one contact perforating gunshot wound to the left chest with bullet injury to the left lung including the pulmonary artery and vein. Associated injuries included 1,200ml bilateral hemothorax. The firearm was a 45-caliber semiautomatic handgun.

The third case (75-year-old) revealed two contact penetrating gunshot wounds to the midline chest with one bullet causing injury to the right lung, heart, vena cava, and aorta and the other bullet causing injury to the liver, thoracic spine, and descending aorta. Associated injuries included a 2,000ml right hemothorax, thoracic spinal cord subdural and subarachnoid hemorrhages, and a 200ml hemoperitoneum. The firearm was a 9mm semi-automatic handgun.

Conclusions: Multiple gunshot wound suicides can help elucidate the potential for meaningful physical activity after sustaining catastrophic injuries to vital organs. These cases can help law enforcement and forensic personnel understand the amount of time that can elapse from wound to loss of cerebral function and the ability of an individual to perform meaningful physical activity. The cases presented here highlight the extraordinary human capacity and a desertion of the primal nature for survival.

Suicide, Firearm, Multiple Wounds

G47 Use of Therapeutic Intravenous Catheters in Drug Addiction: A Series of Three Cases

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After attending this presentation, attendees will become aware of a potentially overlooked deadly method of intravenous drug use. This poster will provide the attendees with a review of the stigmata of clinical and histopathologic findings associated with illicit drug use using therapeutic intravenous catheters.

This presentation will impact the forensic science community by bringing attention to an easily overlooked method of intravenous drug abuse, especially in the hospital setting.

Numerous creative methods and behaviors for abusing prescription and illicit drugs have been detailed in the literature over the years, including the recent tendency to inject or snort crushed prescription medications that are designed for oral ingestion. Numerous articles and news stories have discussed deaths of debilitated people injected by lethal doses of medication or poisons by caretakers, often in hospital settings; however, there has been little discussion of the use of therapeutic intravenous catheter ports in the addicted population. This presentation will discuss two Medical Examiner (ME) cases and one hospital-based autopsy case in which this behavior was reported in the patient's history and/or was shown to contribute to the patient's demise.

Case Description: Case one was an ME case involving a 26-year-old female undergoing chemotherapy for recurrent Hodgkin's Lymphoma who died of acute morphine toxicity. A prescription for morphine sulfate was filled two days prior to her death with only 93 of 120 tablets remaining in the bottle. Investigators at the death scene were suspicious that she had accessed the port of her chemotherapy line for the purpose of injecting her oral medications. At autopsy, microscopic sections showed numerous intravascular and perivascular granulomata with polarizable foreign material, with additional foci of polarizable material found within the splenic and hepatic macrophages. Case two was an ME case involving a 47-year-old female with end stage renal disease secondary to Goodpasture's syndrome who died of acute cocaine toxicity. The investigation revealed she had a history of accessing her port for administering illicit substances. Postmortem toxicology revealed the presence of cocaine and its metabolite in her blood. Case one and two demonstrate the difficulty in managing drug addiction in the chronically ill and palliative care populations.

Case three was a hospital-based autopsy of a 38-year-old male with a history of polysubstance abuse who was admitted for multilevel epidural spinal abscess with cultures positive for *Staphylococcus aureus*. His condition was attributed to intravenous drug abuse. Treatment for his abscess required intravenous antibiotics, so an intravenous portacath was placed. During treatment in the hospital, he was encouraged to ambulate and often left the hospital floor on his own. Some of these excursions were followed by episodes of respiratory desaturation and decompensation leading to extensive workups to rule out a pulmonary thromboembolus. For this reason, clinicians obtained consent for a hospital-based autopsy. At autopsy,

microscopic sections of the lungs revealed much polarizable crystalline material both in the lumens and within the walls of vessels accompanied by granulomatous inflammation with multinucleated giant cells. Some of the vessel walls were thickened due to myofibroblastic proliferation around the foreign material; changes consistent with his two-week stay in the hospital.

Summary: These three cases represent a method of intravenous drug abuse in an addicted population leading to death that is most likely underreported due to lack of recognition, especially when the death occurs in a hospital setting. Chronically ill and palliative care patients, particularly those with terminal illnesses, may not trigger an extensive investigation including autopsy since death may be easily ascribed to the natural disease process. Clinicians working with patients who have addiction problems should consider the increased potential of drug-related fatalities if these patients have in-dwelling intravenous catheters. A closer review of these types of cases is needed to properly assess the true incidence of this behavior.

Pulmonary Granuloma, Intravenous Catheter, Intravenous Drug Use

G48 Homicide, Suicide, or Accident? An Unusual Case of Hanging Attempt

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After attending this presentation, attendees will be able to understand that sometimes the evidence does not necessarily correspond to what we think. Crime scene investigation needs a skeptical approach to evaluate the better way all the evidence is useful to establish the manner of death.

This presentation will impact the forensic science community by demonstrating the importance of a multidisciplinary competence in crime scene investigations that need a well-established methodology by forensic pathologists rather than emergency physicians or policemen that arrived before them and could misrepresent the evidence and autopsy findings in differential diagnosis between self-inflicted hanging and blunt lesions.

Hanging has many features in common with ligature strangulation. Death is, however, more often caused by reflex cardiac arrest from pressure on the carotid structures. It is difficult to evaluate the survival time after hanging; hence, if death is not sure, the physicians have to cut the rope to start resuscitation. Hanging's victims are generally found with pale faces, rather than the congested, hemorrhagic appearance of the slower asphyxia type of death. Most hangings are self-suspension. This may be carried out by a wide variety of methods, but a typical method of self-suspension is to attach a thin rope to a high point, such as a ceiling beam or staircase. The lower end is formed into either a fixed loop or a slipknot, which is placed around the neck while the intending suicide stands on a chair or other support.

The case presented is of a 32-year-old man who was found suspended from a balcony in a courtyard. Police and Emergency Medical Service (EMS) was called immediately but, physicians believed the man was dead when the crime scene investigation started. The right hand of the victim was grasping a node on the right side of the neck and the left hand was grabbing the unstable loop knot. After few minutes, the body fell to the floor and the ear started bleeding.

At external examination, a late-onset right orbital hematoma was found, suggesting a fracture at the skull base. The occipital region of the head showed a star-shaped lacerated wound. A typical hanging mark was evident around the neck, not completely encircling it because of the two gaps at the sides of the neck, for the interposition of fingers between noose and skin. The autopsy showed brain epi and subdural hemorrhage with skull base fracture. The histological analysis of the lung tissue showed alternating areas of alveolar collapse and overinsufflation by compensatory emphysema, typical of a slow asphyxia. No alcohol or drugs of abuse were found in the blood and urine collected at autopsy. All these findings demonstrated that the cause of death was due to traumatic cranio-cerebral injury which occurred when the subject was still alive, even if in agonal state following the hanging. Circumstantial data revealed that the victim was homosexual, and he was falling out of love with his boyfriend. In the past, he simulated other self-inflicted hangings, the last one occurring two days before his death.

The case was closed as an accident consequent of a suicide attempt. The manner of death and focus on possible liability of EMS personnel in determining death was speculated.

Hanging, Suicide, Crime Scene Investigation

G49 Cell Death Proteins as Markers of Early Postmortem Interval

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After attending this presentation, attendees will consider the possibility of studying early postmortem interval based on the biochemical events related with cell death.

This presentation will impact the forensic science community by introducing a potential quantitative indicator of postmortem interval in the first hours of death, as well as the reliability of using mRNA toward this purpose.

Mainly, the estimation of postmortem interval is analyzed from one day to years after death, using multidisciplinary approaches. A panel of physical methods, such as body temperature, and thanatochemistry methods, like potassium or amino acids measurements, were the subject of further studies and seem to be useful in combination with other factors. However, there have been few reports on RNA expression in a dead body. These studies have evaluated mRNA stability using human housekeeping genes, finding a correlation between these parameters.

Decomposition begins approximately four minutes after death with a process called autolysis. It is a cell necrosis, similar to the process induced when an organ suffers isquemic or anoxic alterations. During the decomposition, the cells will be progressively destroyed. As a consequence of that, there is a release and damage of cellular components and metabolites, which induces inflammation and cell death. However, the nucleus remains without alterations until four days after death, which will permit applying molecular biology methods in order to determine time-since-death.

This research was to study early postmortem interval, between two and eight hours, using the analysis of the expression of two dead/inflammatory proteins, FasL and PTEN, by Quantitative-PCR and assess reliability of this method for mRNA in specimens from dead bodies.

Four adult male Wistar rats (250-300g, aged three months) were euthanized at the same time with intraperitoneal injection of 0.15ml/Kg Rompun® (Bayer). Immediately after death, as a time zero or control, 20mg of gastrocnemius muscle were biopsied from each rat. The rat bodies were placed in the laboratory at room temperature. From two until eight hours after death, 20mg of this muscle were collected bi-hourly. The samples were homogenized and total RNA was extracted using GenElute Mammalian Total RNA Miniprep Kit (Sigma), according to the manufacturer's protocol. The RNA was quantified using NanoDrop 2000c (Thermo Scientific, NanoDrop Products, Wilmington). RNA was subjected to reverse transcription using High-Capacity cDNA reverse transcription Kit (Applied Biosystems), according to the manufacturer's protocol. Quantitative analysis of FasL (implicated in death cell signaling and inflammation) and PTEN

(inhibitor of PI3K/Akt pathway, which promotes cell proliferation) mRNA levels was performed by the SYBR Green Real Time PCR. Each sample was tested in triplicate. The analysis of relative gene expression data was calculated using the $2^{\Delta CT}$ method.

A total of 20 muscle samples were obtained over an eight-hour period. RNA quantification showed variability between subjects and times, ranging concentrations between 1-25ng/µl; however, this amount of RNA was enough to perform reverse transcription.

Quantitative-PCR results were similar in the two genes. There was a time-dependent increase in the mRNA levels of FasL and PTEN until six hours after death. Though, at eight hours, the levels of these two genes decreased, probably due to the degradation of RNA as a consequence of the progress in the autolysis process. Using regression analysis in the first six hours after death, a positive linear correlation was found between the mRNA expression of these genes and the time-since-death. These results are in agreement with the initial hypothesis, since FasL and PTEN are implicated in cell death and inflammatory signaling pathways.

The findings from this research provided a new quantitative tool for estimating early postmortem interval based on the biochemical events of the autolysis process, even though it could not be estimated past six hours after death due to degradation of RNA. Future research may be able to expand on these results, looking for other cell death markers and extending time-since-death estimates.

Postmortem Interval, Cell Death Markers, mRNA

G50 The Application of Flow Cytometry as a Rapid Screening Method of Samples of Vitreous Humour to Avoid Miscalculation of the Postmortem Interval

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After attending this presentation, attendees will understand the confirmation and quantification of possible accidental contamination with blood of samples of vitreous humour by means of applying the flow cytometry method to the estimation of the Postmortem Interval (PMI).

This presentation will impact the forensic science community by demonstrating the susceptibility of vitreous humour to blood contamination and how this can affect PMI estimates. The determination of PMI is a critical issue in forensic science and methodologies based on the biochemistry of the vitreous humour can provide good results.

In the field of Legal Medicine, the correct determination of the time of death is of crucial importance due to its criminal and civil repercussions. In recent years, research into maximizing the precision and reliability of this estimation has been a recurring subject of investigation, and it is of utmost importance to have recourse to a method that can provide this information quickly and accurately. The best results for estimating time of death derive from the biochemistry of the Vitreous Humour (VH) and are based on examining the relationship between PMI and increases in K+ and hypoxanthine (Hx) levels. Latest reports in the literature suggest that the manner of death can also modify this relationship and computerized programs which take into account these variables have been published. However, contamination by causes not readily apparent, such as blood from the accidental puncture of blood vessels, can occur, and thus lead to an erroneous estimation of PMI.

Objective: The goal of this presentation is to confirm and quantify blood contamination in samples of vitreous humour by using the Flow Cytometry method which reveals how this contamination substantially alters the estimate of the PMI.

Method: Vitreous humour samples were obtained from fresh bodies, and artificially contaminated with known concentration of human blood (5µl of 10% blood in 300µl of HV) and serially diluted in FACSflow™ (Coulter) in order to determine the minimum amount of erythrocytes detectable and its relation to hypoxanthine quantification.

Samples were run in a Facscalibur cytometer (Coulter) at medium flow (12µl/min) and erythrocytes detected in an FCS vs. SSC density plot.

The detection was considered valid when the detected number of erythrocytes stood at the theoretical logarithm ± 0.3. Greater dilutions showed values closer to background noise.

Determination of Hx by LC-MS/MS: A Quattro Micro tandem mass spectrometer (Waters) was employed for the detection of Hx. Chromatographic separation was performed using an Atlantis T3 (2.1x100mm, 3µM) analytical column, working in gradient mode, with acetonitrile and ammonium acetate 10mM (pH=4.5) as mobile phase. Two precursor-product transitions per compound were monitored except for Internal Standard (IS).

Results and Discussion: This contamination noticeably affects the result, and thus emphasizes the importance of careful sample extraction. The cytometric method was able to detect erythrocytes in 1:1250 dilution of contaminated HV. This represents a presence of 6,000 erythrocytes per ml, much lower than that detectable by microscopic counting. This concentration is 0.0017µ| of blood in 1 millilitre of HV.

The sensitivity of the technique, its widespread distribution in the laboratories, and the speed of the results recommend it for the rapid screening of samples.

Postmortem Interval, Vitreous Humour, Flow Cytometry

G51 DNA Sequencing for Identification of Funambulus Species With Possible Forensic Implication for Conservation of *Ratufa Indica Elphinstoni*

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After attending this presentation, attendees will be aware how poaching cases of endangered species are handled in India.

This presentation will impact the forensic science community by increasing knowledge on wildlife DNA for forensic work.

Wildlife forensics is a relatively new field of forensic science. Wildlife forensic scientists must be prepared to identify evidence from any species in the world that is illegally killed, smuggled, poached, or sold illicitly. Despite the frequency of poaching, the perpetrators are seldom charged with breaking the law; even more rarely are such individuals caught in the act of transgression. The modern advent of molecular DNA forensics now adds a new dimension to wildlife law enforcement and may, eventually, also serve as an added deterrent. Molecular data is sometimes the only type of information available to conservation officials in their field investigations. The latest DNA-based technologies today make it feasible to identify single individuals by DNA typing from only trace amounts of their genetic material. The application of DNA technologies in wildlife forensics demands not only that gender and individual profiling be determined, but that the target animal species be also identified correctly.

The Indian giant squirrel, *Ratufa indica*, is a large-bodied diurnal, arboreal, and herbivorous squirrel found in South Asia commonly known as “Shekru” (*Ratufa indica elphinstoni*). It is the state animal of Maharashtra. It inhabits in the deciduous or mixed forests and is abundant in the forests of the Western Ghats of Maharashtra. The animal is protected in Bhimashankar Wildlife Sanctuary. *Ratufa indica*

elphinstoni is a conservation priority for the State of Maharashtra in India. It is imperative to secure *Ratufa indica elphinstoni* populations in all the areas they currently occupy, whilst encouraging expansion into some of their former range. The identification of the species becomes an essential part in forensic work in cases involving Wildlife Protection Act (1972) cases.

DNA sequencing allows unequivocal identification of species in forensic cases. Molecular techniques such as DNA barcoding are gaining importance in recent years to resolve these questions. Here in this study, we have checked the utility of DNA barcoding for species identification of *Funambulus* and *Ratufa indica elphinstoni* using mitochondrial genes Cytochrome Oxidase I (COI) and two ribosomal genes (12S and 16S). This analysis successfully discriminated both the species.

When identification of species is essential in forensic wildlife cases, the above technique can be used unequivocally for identification of these species.

The use of animal DNA evidence in forensic investigations is a new and emerging field. In particular, the modern DNA-based molecular methods will aid in the fight against the poaching of endangered and protected species, and in the prevention of cruelty to animals. The illegal culling, collecting, and trading of animals (and animal products) can now be revealed more effectively, and a link between the victim and the suspect established with a far greater degree of confidence. The continued development of a standardized set of protocols for wildlife forensics will further enhance the capacity of law enforcement officials to protect and conserve animals in the wild.

Species, DNA, Forensic

G52 Cotton Swabs vs. 4N6 FLOQSwabs™: A Comparative Study for Optimal Recovery of Touch DNA

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After attending this presentation, atattendees will have a better understanding of how touch DNA recovery could vary between different collection swabs, swabbing techniques, and extraction processes, and how different surfaces affect touch DNA recovery with two different types of swabs: 4N6 FLOQSwabs™ and cotton swabs.

This presentation will impact the forensic science community by discussing the comparison of the 4N6 FLOQSwabs™ and standard cotton swabs used in crime labs in their ability to recover touch DNA from various surfaces as well as the comparison of their ability to preserve touch DNA in the presence of common environmental contaminants.

Over the last few years, the boost in sensitivity and robustness of forensic STR kits has increasingly made touch evidence a potential source of DNA profiles that can become useful to an investigation. Thus, the ability to properly collect and handle touch DNA plays an important role in this process, given the small amount of genetic material left behind from handling an object, mostly from epithelial cells. Standard practice in many laboratories is the use of moistened cotton swabs to swipe the surface allegedly touched by the suspect. These swabs are made of cotton fibers wrapped around the tip of a ~ 6" stick. Although highly absorbent, this type of swab has an inside core that can trap cellular materials within its fibers. The 4N6 FLOQSwabs™ are instead made by applying glue to the tip of an applicator and perpendicularly spraying onto this surface tens of thousands of short nylon strands. Liquid absorption is obtained by capillary action induced by the surface tension between the nylon strands. Furthermore, 4N6 FLOQSwabs™ lack an inside core to trap cellular materials.

For the purpose of this study, a common sterile, DNAase/RNAase-free cotton swab was compared to the 4N6 FLOQSwabs™ manufactured by Copan Italia specifically for forensic applications. The FLOQSwabs™ are produced in a controlled human-free environment and are certified human DNA, DNAase and RNAase-free, are treated with an antimicrobial reagent, and undergo ethylene oxide sterilization; they have a breaking point right above the swab tip to facilitate the transfer of the swab inside the Nucleic Acid Optimizer (NAO™).

Touch DNA evidence was simulated by having volunteers handle specific objects for one minute (glass, wood, and leather). The handled objects were divided into quadrants and swabbed with the two different types of swabs in duplicate. Multiple experiments were performed and different combinations were tested: multiple individuals, different swabbing techniques (wet vs. dry), and the use of a NAO™—a basket with a semi-permeable membrane) that is used during extraction. The experiments were designed in order to standardize conditions as much as possible and ensure that the amount of cells on the various quadrants were similar; nevertheless, the amount of cells that are shed by an individual is somewhat unpredictable. Thus, to determine the actual percentage of DNA recovery, known amounts of DNA were spotted onto glass slides and allowed to dry. Slides were then swabbed with the two different types of swabs and subjected to extraction (DNA is basically being re-extracted). Due to the expected loss of DNA during the re-extraction process, results were normalized against the same amount of DNA re-extracted directly from the tube.

Results show that, on average, 4N6 FLOQSwabs™ yielded greater amounts of DNA than the cotton swabs, particularly when sample collection was performed with the moistened swab and extraction was conducted with the aid of the NAO™. This presentation will also discuss how the antimicrobial treatment protects the DNA from bacterial degradation after sample collection.

Touch DNA, Cotton Swab, Flocked Swab

G53 Some Like It Extra-Dry: Specific Skeletonization Patterns Due to Larder Beetles (*Dermestes spp.*)

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After attending this presentation, attendees will understand how the presence of larder beetles (*Dermestes spp.*) may affect the decomposition and skeletonization processes. Through the years, several authors reported bodies discovered in flats or houses with thousands of larvae and adults actively feeding on them. However, these reports are scattered and, no study addresses this question from both anthropological and entomological point of view.

This presentation will impact the forensic science community by summarizing the cases reported in the literature, by establishing the skeletonization patterns caused by larder beetles and by offering a PMI estimation process based on adults, larvae, and molts abundance.

In a dry and warm environment, skeletonization usually occurs in a few weeks. When fly larvae (maggots) are present, they feed on soft tissues, especially the eyes or mouth. The presence of hundreds of larvae actively feeding on a corpse may thus result in a quick skeletonization of the face; however, necrophagous fly' larvae avoid hard or dry tissues, and thus do not contribute to the skeletonization of feet or hands. On the contrary, larder beetles feed on dry tissues.

Dermestes spp. (larder beetles) are coleoptera species frequently observed on dry human remains. These species are well adapted to exploit all kinds of dried animal tissues: skin, fur, leather, bones, etc. They are widespread among fish and meat smokehouses or saltering, in poultry houses (due to the presence of manure), or even natural history museums. Some decades ago, they were

common pests of stored food products or tanneries. Hundreds of species have been reported, but only five to ten of them are commonly observed on human remains. As these species often travel with food or furs, they have a worldwide spread.

The adult female laid several egg-batches of two to twenty eggs (depending on species) in cracks or cavities of the substrate. Both adults and larvae avoid bright light. Larvae feed on surface, but burry themselves for each molt. They have seven or eight molts before pupation. Just before pupation, larvae stop feeding and move away to find a suitable place to pupate. After several days of pupation, adults emerge and start again to feed. Under favorable conditions, *Dermestes* are rapid breeders. Depending on temperature and species, an entire generation may be developed in five to six weeks. During her life, a female can lay more than 200 eggs. So, in warm conditions and with sufficient food available, *Dermestes* species may have more than five generations annually. In such cases, it is common to observe on the same place a high number of adults, larvae, molts, and frass (i.e. fecal material wrapped in peritrophic membrane).

In a forensic context, *Dermestes* (adults or larvae) are usually found from active decay phase until skeletonization. However, they are especially abundant on dry remains. They first remove flesh from limbs and face. This feeding pattern produces a quick and quite specific skeletonization of the extremities. Furthermore, larvae excrete frass in abundance. Together with molts, these residuals can create a substantial amount of material around or even inside the corpses. This is especially true for long Postmortem Interval (PMI) indoor cases, when victims are discovered several months after death. Lastly, due to the possible feeding of *Dermestes* larvae on empty puparia or dead flies, the former presence of Calliphoridae species is often difficult to assess. However, the time needed to complete several *Dermestes*' development cycles can be calculated depending on ambient temperature and species.

To be answered, the PMI estimation of such cases needs the highlight of both a forensic anthropologist and entomologist. Working together, they should be able to interpret the skeletonization and the abundance of insects to estimate the time of death.

Long PMI, Indoor Cases, Entomology

G54 Estimation of Postmortem Interval by Direct Analysis of the Skin Surface Through FT-NIR

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After attending this presentation, attendees will be familiarized with the basics of Fourier Transform-Near Infrared (FT-NIR) spectroscopy and multivariate analysis using common chemometric techniques such as Partial Least Squares (PLS) and Principal Component Analysis (PCA). In particular, the audience will learn about the potential for creating models for the estimation of the Postmortem Interval (PMI) when joining chemometrics and NIR spectroscopy. General ideas for the application of such techniques to other forensic problems may be also addressed.

This presentation will impact the forensic science community by introducing modeling techniques over NIR spectroscopy to the problem of estimating the postmortem interval in a fast, reliable, and rather accurate method. Besides, the procedure proposed requires minimal to none sample treatment or preparation and can be performed *in situ*.

The estimation of the PMI, the time since death, is of paramount importance and a significant challenge for the pathologists. Methods currently employed have considerable inaccuracy.

Following the developments of the last few years regarding the application of mid-infrared spectroscopy for studying skin, and the possibility of easy direct *in situ* analysis that has risen with fiber optics apparatus for Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR), the FT-NIR was applied spectroscopy to directly test the human skin for possible chemical changes occurring after death that could correlate with PMI.

The sample population consisted of 25 corpses (male and female) with different causes of death and ages ranging from 20 to 96 years old. The bodies were submitted to autopsy at the North Branch of Portuguese National Institute of Legal Medicine and Forensic Sciences and the spectra recorded at the premises by the local staff. FT-NIR spectra have been acquired using an Antaris 1 (Thermonicolet) spectrophotometer equipped with a diffuse reflectance fiber optical probe (SabIR, Thermonicolet). Spectra (in duplicate) were taken from the upper right lateral toraco-abdominal area and internal side of upper third of right arm at each postmortem acquisition point and the changes in skin chemistry were followed during time periods of 9 hours (minimum) to 69 hours (maximum), depending on the subject, until a maximum of 72 hours.

Several chemometrics techniques were applied for the multivariate analysis of the spectra, namely PCA, PLS, and Partial Least Squares Discriminant Analysis (PLS-DA). All modeling and data analysis was done using Matlab (version 7.9, Mathworks) and PLS toolbox 5.5.1 (Eigenvector Res1earch Inc.) for Matlab. On creating the model, specific functions were written for variable selection, using genetic algorithm, and for sample selection for cross-validation.

The models obtained, although having a restricted number of corpses available for calibration, present promising results.

Postmortem Interval, Infrared Spetroscopy, Skin Chemestry

G55 Autopsy Approach in Forensic Investigation of Child Abuse

Agostinho Santos, MD, PhD, Katerina Puentes, MSc, Patrícia Jardim, MD, and Teresa Magalhães, MD, PhD, Univ of Porto, Jardim Carrilho Videira, Porto, PORTUGAL*

After attending this presentation, attendees will hear standardized recommendations followed by investigative procedures and data collection instruments in child abuse fatality autopsies at the North Branch of the National Institute of Legal Medicine and Forensic Sciences in Portugal, as well as contributing to a new approach in these types of autopsy by standardizing methods and procedures.

This presentation will impact the forensic science community by showing the importance of standardization of investigative procedures in forensic investigation of fatal child abuse situations. This type of investigation must be as complete and thorough as possible, and ideally, observe standardized procedures. It must also be executed by experienced professionals in the area of child abuse who are able to adjust and adapt to standard techniques for every specific situation.

Although a high-detail skeletal survey reported by a radiologist is mandatory in order to identify and record skeletal injuries suggestive of or consistent with abusive situations, imaging techniques may sometimes over or under diagnose some of these injuries. Taking this fact into consideration, in fatal child abuse situations, disarticulation and removal of the entire costal grid, followed by a maceration process for a non-traumatic soft tissue removal may be very useful for an adequate documentation of any skeletal fractures (ribs and sternum) with minimal artifact formation.

Methods: Internal examination begins with the incision of the scalp, either through a bi-mastoid incision, or a posterior approach. A "stem-to-stem" double Y incision at both the anterior and posterior aspects of the trunk is a fine approach to initiate internal examination of neck, thoracic, and abdominal structures. Incisions performed in cases of fatal child abuse should allow for optimal visualization of the underlying structures and eventual lesions, without causing, however, a great negative visual impact. All major cavities, cranium, thorax, and abdomen, should be examined and dissected "layer by layer" and whenever is necessary, special autopsy procedures should be applied, such as eye and middle ear removal. Removal of the entire

thoracic cage includes the spine, from C5 to L2, clavicles, the sternum, and all ribs, keeping all the bony structures completely articulated between them. Soft tissue elimination and disarticulation of the bony elements is achieved through a maceration process with enzymatic detergent. Careful reconstruction of thorax morphology with prosthetic devices followed by proper suture of all incisions allows the performance of this technique without causing, however, a negative visual impact on the body.

Results and Conclusions: The implemented procedure allows a neat observation in order to rule out recent and non-recent fractures with minimal artifact formation due to instrumentation otherwise caused by standard autopsy techniques and without causing a negative visual impact on the body.

Child Abuse, Skeletal Trauma, Autopsy

G56 "Hot Shots": A Forensic Approach to Allegations Surrounding Fatal Consequences of Injected Substances of Abuse

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After attending this presentation, attendees will have an awareness of the pervasive and variable concept of "hot shot" in the non-forensic community. A working definition for forensic scientists is suggested. Attendees will also be able to describe an approach to autopsy that will optimize the potential to refute or support the allegation or at least demonstrate that all due consideration has been afforded the possibility.

This presentation will impact the forensic science community by highlighting the lay concept of "hot shot" and how pathologists can address the allegation.

The term "hot shot" is variably defined and is well-ensconced in the public consciousness. Although it may encompass accidental death due to an unexpectedly high drug concentration, it more commonly implies malicious involvement of another and homicidal intent. Even a brief perusal of lay websites reveals awareness that benzodiazepines will potentiate opiates, and creative and alarming suggestions to make injections painful or fatal. Typical perceived victims are informers, fellow addicts (for robbery or revenge), or targets of organized crime. The concept appears regularly as a plot device in popular media, in the news, and as propaganda.

The concept has been neglected in forensic literature. Although the pathologist is rarely able to support or refute it, there is a need to acknowledge and define it, develop an approach to examination, and establish an academic data base. Complacency may result in distress for families, dissatisfaction on the part of law enforcement/prosecuting attorneys, public concern, embarrassment, and fostering of conspiracy theories.

A search of the database of DOFM Glebe revealed no cases of injected drug toxicity or detected adulterants that would reasonably represent homicide. Cases have gone to inquest on such allegations, but none appear to have had sufficient evidence to support taking the matter to trial. Law enforcement and the media in this district nevertheless regularly assert that a "hot shot" has occurred.

It has been suggested that a threatened victim would sustain obvious defense injuries; the usual scenario proffered, however, is a non-naive intravenous drug user unaware of the danger. A past history of drug abuse tends to suggest recidivism rather than homicide.

The first step in allaying concerns is to elicit what the concept means to the individual raising the assertion. The following is a suggested forensic definition of "hot shot": The *deliberate* injection or provision of materials for injection of an *abused substance* that is known to *likely be lethal* due to extreme purity, increased concentration, or adulteration by a known poison.

Although allegations may be misguided, the truth is that street drugs are invariably adulterated to "cut" the active substance, improve the appearance of the product, and introduce toxicological "distractions" (i.e., local anaesthetics). Drug substitution may occur (i.e., heroin for cocaine). There have been outbreaks of acute reactions to pharmaceutical adulterants (i.e., clenbuterol/scopolamine). There may be contaminants, including bacteria (with outbreaks of anthrax and Clostridial disease) and heavy metals (i.e., lead). Adulterants may have deleterious effects that contribute to death, including drug toxicity, non-drug poisoning, sepsis, and allergic reactions.

Identification and medicolegal verification of a "hot shot" is predicated on a thorough investigation, ideally with information regarding the nature of the presumed fatal injection. It also requires the collaboration of a toxicologist, possibly with the task of identifying an unusual substance.

In cases of alleged "hot shot" prior to autopsy, an examination that is arguably more thorough than the usual "overdose" case should be considered; the goal is to gain as much information as possible as to the causes and circumstances of death, bearing in mind that even negative findings of violence may help to clarify the situation. The allegation is usually predicated on the assumption that death occurred quickly, so evidence of a survival time may be important. Features of past drug abuse may be significant in some situations. A list of autopsy procedures for consideration in such situations will be presented.

Forensic pathologists need to be honest with law enforcement, prosecutors, and families about the limitations of autopsy in these cases, but acknowledge the possibility of homicide and approach the autopsy with thoroughness and an open mind.

Hot Shot, Drugs of Abuse, Homicide

G57 Sudden Cardiac Death of a High-Performance Skater

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After attending this presentation, attendees will understand how the misuse of Over the Counter (OTC) anabolics alter the cardiac architecture, and is a predisposing factor for sudden death. Another important fact is that the investigation of prior history associated with an autopsy-oriented procedure led to establishing an association between inappropriate use of these drugs with a viral infection, which led the deceased into a lethal pathophysiologic event that could happen again under the same circumstances. Since sudden deaths of athletes commonly involve drug abuse, a toxicology screening was ordered for fluids like blood or urine of the deceased, with negative results. The histopathology results were reviewed by departmental forensic pathologists and academic peers of an associated teaching hospital. It is believed that this case of accidental death was never reported.

This presentation will impact the forensic science community by understanding how multidisciplinary research collaboration involving the drug manufacturer and the system of distribution, toxicologists, and forensic medical team was able to reconstruct the chain of events in an appropriate manner. Determining the factors that influenced this unusual death was difficult at first, but the main purpose in determining the manner of death was because it created a controversy between the authorities who removed the corpse from the scene, the family, and the forensic pathologists because the absence of significant medical history, even the use of narcotics or anabolic illegally manufactured were considered. So far, the manner of death has not been determined. The products involved are OTC and do not require a medical prescription for sale. Health systems in Colombia, through an entity called the INVIMA regulator for the distribution of drugs (equivalent to the FDA), have not been given a decision on

restrictions on the use and sale of such anabolic in gyms, pharmacies, and sports shops.

Another sudden cardiac death case was reported in a high-performance skater who suddenly collapsed during a training session. This report examines the medical history, the chain of events, and pathogenetic mechanisms leading to death. Medicolegal aspects of the case are discussed after available literature is reviewed. There are a number of articles reporting many cases of sudden cardiac death in athletes who use performance-enhancing drugs, but there are no reports regarding this type of death where the use of OTC anabolics is involved as a contributing factor to death.

The last unusual sudden death case presented is associated with the consumption of OTC anabolics. This case was studied and used as an example of the practical application of research and multidisciplinary collaboration for the discovery of an unusual death. It is recommended that forensic investigators are familiar with these types of drugs which are common among athletes and the pathogenesis of this way of death that may have involved an inadequate response from a heart changed because of a viral infection.

Sudden Death, Cardiomyopathy, Anabolic Steroids

G58 What Is That Little Yellow Fragment? Application of Confocal Raman Microscopy in the Identification of the Means in a Case of Murder by Blunt Object

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The goal of this presentation is to report a case of homicide on a 42-year-old man who was found dead in fields around Foggia, close to a blood-stained brick. After attending this presentation, attendees will understand, in order to identify the tool used in case of homicide, the importance of analyzing and integrating the data derived from a well-detailed crime investigation, autopsy examination, and subsequent histological investigations, as well as using a new spectroscopy technique applied to microscopy called confocal Raman.

This presentation will impact the forensic science community by demonstrating how investigation and research resulted in the conclusion that the tramma to the victim was caused by several weapons.

Finding compatibility between the cause of death and the tool used is a hard daily routine task for the forensic pathologist and not easy to achieve. Often the wounds found on the corpse may be compatible with several tools, so the crime scene data might not be instrumental for the identification of the tool used. A crime scene investigation was performed by a forensic pathologist and the police who found a 42-year-old Caucasian man dead in the fields around Foggia. The corpse was lying supine on the grass, with the head fully bloodstained and bloodstained banknotes in his mouth. The head and face showed numerous blunt wounds. Close to the head the grass was covered in blood, and near the corpse was a brick measuring 40x20x20cm, smeared with blood. Nothing else was found at the crime scene.

A multidisciplinary forensic approach, which included CT scan analysis, autopsy, histological investigation, and confocal Raman microscopy analysis was performed. The external examination revealed the presence of multiple lacerations of the scalp in the fronto-parieto-temporal, with several exposed fractures of the skull. Also, other lacerations of skin were shown in the face with a fracturative complex of bones. Furthermore, the neck presented multiple abrasions and purple bruises. The CT scan analysis of the head demonstrated multiple fractures of neurocranium and facial bones, in particular of the skull in the left fronto-parietal.

The autopsy examination confirmed the multiple fractures of the skull and the face, in the parieto-frontal side and the maxillary bone. The detailed examination of the skull fractures at the left fronto-parietal detected a little yellow fragment of unknown material which wasremoved for subsequent exam with confocal Raman microscopy directed to identify its molecular structure. In addition, the internal examination revealed multiple contusions of the brain, especially in the left parietal side and at frontal level. The examination of the neck revealed hemorrhagic area of sterno-cleido-mastoidei muscles, and also the fracture of the thyroid cartilage with contusion of thyroid, along with the presence of blood inside trachea. No other traumatic injuries were found. The microscopic findings were represented by polyvisceral stasis, widespread and extensive cerebral intraparenchymal hemorrhages, cytotoxic and vasogenic edema, massive pulmonary edema, acute emphysema and atelectasis, and blood aspiration. According to the crime scene data, autopsy, histological, and CT scan findings, the cause of death was attributed to multiple skull and face fractures with deep brain contusions, blood aspiration, and combined mechanism attributed to an external neck compression suggested by the fracture of the thyroid cartilage.

With regard to the blunt object used, the results of the forensic investigation pointed out that the lesions revealed on the corpse were linked to the brick found on the crime scene. However, the yellow fragment of unspecified nature, found in correspondence of the skull fracture in the left fronto-parietal, suggested that a tool other than the brick found on crime scene caused this lesion. Therefore, the fragment was analyzed with the confocal Raman microscope.

The Raman spectroscopy is a technique which enabled the discovery of to discover information on the molecular composition of materials and, then, to identify their nature. This technique is based on the diffusion of a monochromatic radiation incident on the surface of an object, which can be absorbed, reflected, or diffused, and it is an ideal tool for identifying and distinguishing between organic and inorganic molecules and crystals. Specifically, the theory behind the Raman Spectroscopy is based on the inelastic scattering of low-intensity, nondestructive laser light by solid, liquid, or gas sample. Confocal Raman microscopy combines the three-dimensional optical resolution of confocal microscopy and the sensitivity to molecular vibrations which characterizes Raman spectroscopy. In confocal Raman microscopy, the chemical composition of a sample can be imaged by recording the integrated intensity of characteristic Raman lines of the substances involved. Thus, one can investigate whether and to which degree mixtures of substances are homogeneous on the length scale of micrometers and above. Confocal Raman microscopy is a relatively new technique that allows chemical imaging without specific sample preparation. By integrating a sensitive Raman spectrometer within a state-of-the-art microscope, Raman microscopy with a spatial resolution down to 200nm laterally and 500nm vertically can be achieved using visible light excitation.

Confocal Raman Microscopy, Homicide, Blunt Object

G59 Gaze Deviation as Image Evidence of Staged Death Scene

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After attending this presentation, attendees will understand the importance of postmortem gaze in scene analysis and its place in the evaluation of forensic imagery of death scenes.

This presentation will impact the forensic science community by providing a better tool for analyzing images of death scenes.

With the ubiquity of digital cameras, more and more images of forensic interest are obtained not by professional forensic

photographers, but as casual and found images taken by onlookers. In one recent case, a digital image of the scene of a possible homicide was obtained during a search of a house for a different reason. The purported victim was a person with outstanding warrants. The question arose as to whether or not this scene was, in fact, a death scene, or if the scene had been contrived in order to convince investigators to stop searching for the alleged victim. There were multiple issues with the scene involving multidisciplinary evaluation by experts in fields such as bloodstain pattern analysis, forensic pathology, crime scene, and others.

While the investigation is continuing, and the details of this particular scene may not be available at the time of presentation, a number of interesting questions arose involving the positioning and appearance of the body in the images. In particular, an examination of the metadata of the image and of the statistics of the image data suggested that the image had been modified. Content analysis revealed numerous incongruences, one of which was that the gaze of the decedent was directed sharply to the side, away from the camera. It has been the experience of the forensic pathologists involved that, in the absence of head trauma that deforms the geometry of the face, postmortem gaze has always been in neutral position. However, no quantitative data was available to support this conjecture.

To evaluate this, a series of measurements of gaze were performed, using a modification of the Hirschberg test. The Hirschberg test is a common and simple technique, used in ophthalmology to test for strabismus, in which a light is shined onto the cornea and the location of the specular highlight is noted. In patients with neutral gaze and without strabismus, the specular highlight will be minimally to the nasal side of the center of the pupil if the light is directly in front of the eyes. If the gaze is diverted, then the specular highlight will be off-center equally and, if there is strabismus, then one eye will be centered and the other off-centered. There are some logistical issues with performing this test in the autopsy suite. Ensuring proper lighting and camera alignment requires careful positioning of the camera, light, and body. Corneal clouding may be partially alleviated with the use of saline solution or glycerin. Changes in eye shape may occur after death that may make the measurements unreliable.

Preliminary results indicate minimal variation from neutral gaze. This result is not surprising. Numerous studies have been done evaluating changes of gaze with anesthesia; the loss of eye movement, papillary reflex, and neutral gaze are all indications of deep anesthesia (though there may be nystagmus in lighter anesthesia and occasional ventral rolling of the eyes at some levels of deeper anesthesia). It is, thus, not surprising that in death a similar relaxation to neutral position occurs. Gaze deviation of the purported victim in death scene imagery may, thus, provide an indication of staging.

Postmortem Gaze, Image Analysis, Scene Analysis

G60 Limitations of Metal Fragment Analysis on Postmortem Computed Tomography (CT)

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After this presentation, attendees will understand the technical limitations of extended scale Computed Tomography (CT) measurements of density Hounsfield Units (HU) as applied to metallic ballistic fragments.

This presentation will impact the forensic science community by stressing that as postmortem CT is incorporated into medicolegal death investigations it will become necessary to carefully define standards for determination of metal fragment characteristics. If extended scale CT is to be used to determine whether fragments in a body are of the same material or to predict the composition of metal fragments based on density (relative to samples of known composition), the influence of Region of Interest (ROI), size, and placement must be appreciated and standardized techniques developed.

A logical forensic application of postmortem CT is the characterization of ballistic fragments present in the body at the time of medicolegal death investigation. Density measure has the potential to determine if fragments in a body are of the same material and to predict the composition of metal fragments based on density relative to samples of known composition. The purpose of this study was to evaluate the application of standard ROI analysis to extended CT scale measurements of density HU. The goals were to determine the influence of ROI size and placement on the density measure of gunshot fragments.

Postmortem CT imaging of gunshot wound victims was performed on a CT scanner with extended scale. Analysis of imbedded metal fragments was made on a radiology workstation using the standard software tools for measuring HU's from regions of interest. ROI placement and size were varied in assessment of metal fragment density while in the body and repeated in air after recovery at autopsy. Sample rounds of known origin were scanned in water and air for comparison and effects of kVp and mA were evaluated.

Both recovered fragments and bullets of known composition showed highly variable estimates of density depending on ROI area and placement. Tested fragments ranged from 1,200 to 30,000 HU. Values were varied for ROI size and location whether a fragment was scanned longitudinally or transaxially. Fragment measurements in the body produced densities approximately 50% lower than values measured in air. Lower scan kVp produced higher HU values (air measurement). Because of the wide variation in extended scale HU measurements occurring within and between fragments it was not possible to conclude whether fragments, were from the same source material or to distinguish composition of higher density fragments such as lead, steel, and copper.

ROI size and placement affect the CT measurement of density (HU's) in metallic ballistic fragments to such a degree that extended scale determination of density should not be used in forensic analysis without further investigation. Scatter and beam hardening artifacts occurring with present CT algorithms are responsible for these variations. New algorithms or correction of existing algorithms should be considered.

Postmortem CT, Ballistic Fragments, Metal Analysis

G61 Postmortem Lung CT in Hypothermia — A Retrospective Age Sex Matched Study

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After attending this presentation, attendees will have a better appreciation for the forensic pathologists use of postmortem scanning to investigate pulmonary anatomy and pathology.

This presentation will impact the forensic science community by adding a morphological clue to possible hypothermia cases. That finding is a low density of lung tissue in CT scans. This is a radiological correlate of acute emphysema or overinflation of the lungs. Pathophysiologically, this is possibly mediated by a shifted oxygen-hemoglobin dissociation curve in hypothermia.

Methods and Material: Since 2010, in the Institute of Forensic Medicine of the University of Zurich, all bodies undergo postmortem CT full body scanning before autopsy. Group H (hypothermia) was defined to contain all deceased between 2010 and April 2012 who died with the pathological diagnosis of hypothermia. Diagnosis of hypothermia had been based on a range of factors such as being plausible due to circumstances, gastric erosions, or a positive test for ketone bodies in urine and no radiological findings were used at the

time to suggest hypothermia. Control group C was selected by randomly selecting individuals from the same period, in a way that allowed for an age and sex match. Cases with intensive care treatment for hypothermia and with no hypothermia immediately preceding death were excluded. Group C was selected to not contain strangulation, drowning, and macroscopic lung injuries. Axial CT slice images containing the lungs were used for further analysis. Each lungs area was examined in two locations, one above and one below the hilus. The whole lung area up to around 5-10mm underneath the outline of the pulmonary parenchyma was manually marked using software. Hypostatic dense tissue or subpleural bullous emphysema was not marked. The average CT density (HU: Hounsfield units) was then obtained for the marked areas. Readings were obtained twice independently by two readers and subsequently averaged. CT scans were obtained on Siemens CT scanners (Siemens, Erlangen, Germany). SECTRA PACS software (Sectra Imtec AB, Linkoping, Sweden) was used to view the CT scans and obtain CT density measurements. Statistical evaluation was done using the R package (R Foundation for Statistical Computing, Vienna, Austria). Approval of the relevant bodies was obtained, ethical regulations were respected.

Results: Hypothermic bodies contained lungs with significantly lower CT density (-764 +/- 67 HU) compared to age and sex matched controls (-546 +/- 154 HU; Wilcoxon p <0.001). Keeping in mind the restricted scope of the control group (as we excluded a number of diagnoses), logistic regression would predict the hypothetic presence of hypothermia at exceeding 90% for CT lung densities under-780 HU.

Discussion: Hypothermic indivuals might exhibit pulmonary findings of suffocation such as the CT appearance of lower density, quite possibly indicating acute overinflation. This is not an unjustified assumption —at 20 – 22°C, the oxygen-hemoglobin dissociation curve resembles the curve found for carbonmonoxide poisoning with 60% carbon monoxide-hemoglobine blood levels. In hypothermia, ventricular fibrillation or asystole is preceded by a left shift of the hemoglobin-oxygen dissociation curve. Cold temperatures cause oxygen molecules to adhere to hemoglobin more. As a result, tissues cannot take up oxygen as well because hemoglobin does not release it that well. This in turn can cause a range of complications. One of these complications is that a left shift of the oxygen-hemoglobin curve in itself is a factor promoting metabolic alkalosis which in turn might entail hyperventilation but which appears to be counteracted, at least to a degree, by lactacidosis due to tissue hypoxia and by respiratory acidosis (reduced carbon dioxide exhalation). Shallow and slow breathing as well as progressive bradycardia seem to reflect an adapted response, but more likely in individuals whose metabolism has sufficiently slowed down as response to hypothermia. An increase in breathing volume, particularly while tissues and cells are not sufficiently cooled down themselves, might then explain our findings. With that, acute emphysema or overinflation of the lungs could indicate that internal suffocation and, in fact, increased and not decreased breathing volumes may play a role in hypothermic death. At any rate, low CT density of lungs in postmortem CT scans should be investigated as a possible clue for hypothermia.

CT Scanning, Radiology, Forensic Pathology

G62 Postmortem CT Angiography as a New Tool in Medical Education and Clinical Research

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After attending this presentation, attendees will understand the role of postmortem angiography in medical education, advanced medical training, and in clinical research as well as the need for close interdisciplinary collaborations of postmortem and clinical medicine.

This presentation will impact the forensic science community by showing how the performance of a postmortem CT angiography can increase the quality of medical teaching in anatomy and highlight the impact of postmortem perfusion on clinical research and advanced medical training.

In postmortem investigations, radiological cross sectional imaging techniques such as Multi-Detector Computed Tomography (MDCT) have already found a place in many medicolegal institutes. More complex methods such as Postmortem CT Angiography (PMCTA) have been introduced in the last years. In order to increase practicability and reproducibility of this kind of exam, a standardized technique called Multi-Phase Postmortem CT Angiography (MPCTA) has recently been developed. This technique consists of a native CT scan followed by three angiographic phases (arterial, venous, and dynamic phase). The vascular system is hereby investigated in a minimally invasive way by cannulating the femoral artery and vein of one side, connecting these cannulas to a perfusion device and injecting a mixture of paraffin oil and contrast agent before and during CT scanning. Adequate technology for performing this examination, including a specialized perfusion device and contrast agent, has recently been developed in Switzerland. The new technique has already been proofed as being a sensitive tool for detecting vascular lesions and to increase significantly the quality of postmortem investigation when it is combined with conventional CT.

But the need for a reliable radiological demonstration of the vascular system has also increased in the field of medical education and advanced medical training. The goal of this presentation is to show the interest of postmortem angiography as a new tool in medical teaching and clinical research and to underline the importance of close collaboration between postmortem disciplines (legal medicine, pathology, anatomy) and clinical research.

Anatomical education for medical students: Human bodies foreseen for anatomical preparation courses have been examined by native CT, magnetic resonance imaging, and PMCTA using the technique of MPCTA. Thanks to the installation of computer displays in the autopsy-room, medical students are now able to compare the radiological data of the investigated body with the topographic anatomy of the same body during dissection courses. As a rsesult PMCTA, the vascular topography can be studied in detail. After the dissection course, anatomical cross sectional preparations have been fabricated which can be compared to the cross sectional radiological images.

In the context of clinical anatomy, the same radiological techniques have been used in order to develop and validate new diagnostic methods and surgical techniques. In fact, for multiple clinical studies, the knowledge of the exact vascular anatomy which can be explored on human bodies by PMCTA is essential. For other studies, the establishment of a postmortem circulation is of interest. Such a circulation can be obtained by using the equipment which has been developed for medicolegal PMCTA. Once a postmortem circulation has been built up, different surgical techniques can be tested on bodies under nearly "*in-vivo* conditions." Even the

performance of interventional vascular examination becomes possible. Without vascular perfusion, such interventions are impractical due to a collapsed vascular system.

The actual development of postmortem imaging and clinical anatomy leads to an increasing demand of interdisciplinary collaboration between different medical fields. This presentation will point out this need and show possibilities for such collaborations. It also demonstrates that a technique, which has initially been developed for forensic purposes, can gain an important impact on teaching and clinical research.

Forensic Radiology, Medical Education, Clinical Research

G63 A New Application of the Multi-Phase Postmortem CT Angiography (MPMCTA) in Sudden Death Cases

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The goal of this presentation is to examine a new approach to forensic autopsy by performing pre-autopsy Multi-Phase Postmortem CT Angiography (MPMCTA) presenting the results collected at the University of Foggia according to a standardized protocol actually used in an international multicenter study. The performance of the MPMCTA in cases of sudden death will be discussed, proposing a new application of that protocol in these cases.

This presentation will impact the forensic science community by providing information for the necessity of a postmortem radiological examination, also performing CT angiography, especially in cases of sudden and unexpected death.

The postmortem CT has become the most common imaging technique used in the last decade representing a routine approach in forensic investigation. Pre-autopsy radiological imaging can reveal different diseases and wounds, but above all it is a significant tool to explain the cause of death as well as to plan the following autopsy examination. However, in the last years, it was concluded that the CT scan without the injection of a contrast agent provides little information about organic lesions and the vascular system.

On one hand, the forensic pathologist needs to visualize the vascular system obtaining nice images, but on the other hand, it seems necessary to perform exact radiological interpretation by decreasing artifacts due to perfusion and by reaching a complete filling of the vascular system. MPMCTA should become a standardized technique. The technique consists of the performance of a native (without contrast-agent injection) CT scan, followed by at least three angiographic phases (arterial, venous, and dynamic phase).

The angiographic phases consist of the perfusion of the vascular system with an oily contrast-agent mixed with paraffin oil, through the accesses in the femoral artery and vein. Accordingly with this technique the vascular system of the head, thorax, and abdomen can be investigated in detail and in a minimally invasive way mimicking the angiographic study in live people. The three phases (arterial, venous, and dynamic) allow a complete opacification of the vascular system and they could show very little vascular lesions or abnormalities as the exact source of bleeding that could be missed during the autopsy. The examination of soft tissues (musculature, subcutaneous tissue) is also significantly increased.

For this research, angiographic exams performed were reviewed for cases of sudden and unexpected death focusing on cases of Thromboembolism. In these series of the cases, it was observed that the access through the femoral artery and vein according to the technique protocol exclude the visualization of the vascular system under the point of injection where the cannulas are positioned. Here a case of a 53-year-old farmer who suddenly died while working in the fields is presented. No particular disease was known by his relatives. Before the autopsy examination, a postmortem CT angiography was performed which showed a filling defect in the right pulmonary artery. This finding suggested the suspicion of a sudden death due to pulmonary thromboembolism. So in order to detect the origin of the embolus, more CT scan images of the limb where the cannulas were not positioned were taken; however, no abnormalities arose from them. In this way, the angiographic study was completed just with the detection of the filling in the left leg. In the course of the autopsy examination, an embolus was detected in the right pulmonary artery, as confirmed by subsequent histological investigations.

In the meantime, the research of the exact site of thrombosis in the peripheral vessels could provide histological data about the age of the embolus itself, to assess the chronological transformation of the thrombus and to determine the causal relationship with pulmonary thromboembolism as cause of death.

In the following cases of sudden and unexpected death, a new application of the protocol is proposed in which the forensic pathologist has to locate and prepare the axillary artery and the axillary vein on one side to insert the cannulas that are regularly connected to the tube system of the perfusion device. All the other parameters of the standardized protocol were not modified.

In conclusion, the postmortem CT angiography using this new protocol is a useful tool to investigate all the vascular system in the cases of sudden and unexpected death, and on suspicion of pulmonary thromboembolism, it can detect the exact origin of the embolus. At last, the native CT scan and PMCTA have to be followed by conventional autopsy and histological examination of the findings.

Postmortem CT Angiography, Thromboembolism, Sudden and Unexpected Death

G64 Efficacy and Efficiency of Multi-Phase Postmortem CT Angiography (MPMCTA) on Altered Bodies

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After attending this presentation, attendees will understand the possibilities, advantages, and pitfalls of performing a postmortem CT angiography on alterated bodies.

This presentation will impact the forensic science community by demonstrating that altered bodies can undergo a Multi-Phase Postmortem CT Angiography (MPMCTA), no matter if they were victims of extensive multiple trauma or if they are altered due to putrefaction. The interpretation of the obtained images can be performed without difficulties as artifacts are easily identifiable.

Over the years, imaging techniques such as Multidetector Computed Tomography (MDCT) have become a routine in forensic investigations. In order to quantify the degree of decomposition and other forms of alteration on the radiological images, the “Radiological Alteration Index” (RAI) has recently been introduced. This index states the alteration of cadavers by quantifying the presence of gas in the body using postmortem MDCT imaging. This means that the RAI increases with growing presence of gas. High quantity of gas inside of the body can be due to multiple traumas or putrefaction.

During the last years, more sophisticated techniques of postmortem imaging have been developed. In order to assess the vascular system, postmortem CT angiography has been introduced. A recently developed technique called MPMCTA uses a standardized protocol and consists in the injection of an oily perfusate mixed with contrast agent. Consequently, the question arises if MPMCTA is still feasible on altered bodies presenting huge quantities of gas.

The goal of this study was: (1) to find out up to which RAI a body can still undergo the technique of MPMCTA; and, (2) which artifacts can be observed depending on increasing RAI.

From a database containing 270 cases on which an MPMCTA, has been performed, we selected those cases which showed an RAI ≥50 (group 1). In order to create a control group, we selected correlative cases showing same age, sex, and cause of death but with a RAI ≤10. The quality of the native CT scan and each phase of the MPMCTA were than evaluated by a radiologist without specific forensic imaging training and a forensic pathologist with special training in forensic imaging.

In group one, 14 cases of our database could be selected. (Mean RAI = 72.5, Min RAI = 50, Max RAI = 100). The control group (n=14) showed a mean RAI of 2.30 (Min RAI of 0, Max RAI of 9).

By comparing the results, we observed that several artifacts were correlated to the RAI index. In fact, while the control group presented artifacts which are already known for the technique of MPMCTA, we observed additional artifacts in group one. In cases where the RAI was related to multiple traumatic lesions, those artifacts were partial or non-opacification of cervical and intra-cranial vessels. These artifacts were related to major arterial or venous lesions at thoracic or abdominal levels such as rupture of the aorta or the superior or inferior vena cava.

More interesting were artifacts observed in cases where the RAI was increased due to cadaveric alteration (putrefaction). In fact, artifacts which had never been described before were detected. Such artifacts were slight extravasations of contrast agent in the juxtadural space of the medulla, in the orbits, the cortex of kidneys as well as peri-splenic extravasations. Also, in glandular structures such as the thyroid, prostate, and suprarenal glands extravasations were detected. In addition, enhancement of the myocardium could be observed which would be considered as pathologic in cases of fresh cadavers. On the other hand, artifacts due to remaining blood and postmortem blood clots which are observed regularly in cases of short postmortem delay seemed to appear less frequently in decomposed bodies. However, the appearance of gas bubbles in the vascular lumen even after contrast agent injection has been noticed to increase with higher RAI.

The performance of MPMCTA in altered bodies is still possible even with a maximum RAI of 100. The diagnostic value of such an investigation remains pertinent and the exam adds further information to the result of conventional autopsy; however, there are some specific artifacts which are related to RAI, especially in decomposed bodies, which are important to know in order to interpret the radiological data correctly.

Altered Bodies, Forensic Radiology, Angiography

G65 Postmortem Whole Body Computed Tomography of Heroin and Methadone Abusers: First Results

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The goal of this presentation is to detect and to learn about characteristic findings in postmortem imaging in cases associated with heroin or methadone poisoning.

This presentation will impact the forensic science community by showing how Multi-Slice Computed Tomography (MSCT) implemented in the diagnostic work-up algorithm of deaths associated with consumption of heroin and methadone might be a helpful tool to improve the daily work of forensic pathologists.

Illegal drug consumption remains an important issue in forensic pathology. Abuse of and ultimately intoxication with heroin and methadone is a frequent cause of death in young adults. Postmortem CT imaging plays an increasing role in the diagnostic workup in forensic pathology.

The purpose of this study was to assess and to analyze the findings in postmortem, full-body MSCT in victims of deaths where heroin and methadone intoxication played a role.

Routinely performed whole-body MSCT scans of 32 cases of non-traumatic death (16 women; 16 men; median age 38 years; range 26-63 years) who tested positive for heroin or methadone consumption by toxicology were retrospectively evaluated. Whole-body MSCT data were analyzed for pathologic findings as well as the images of an age-and sex-matched control group (n=29, 16 women; 13 men; median age 37.5 years.

Nine of the 32 cases were associated with a consumption of heroin in combination with methadone. In six cases, methadone was found to be the only consumed drug. In four cases, heroin was the only detected drug. In a majority of the cases (56%), a mixture of heroin, methadone, benzodiazepines, cocaine, or amphetamines was found.

Most common findings in the drug cases were: pulmonary edema 22 (69%), distended urinary bladder 14 (44%), cerebral edema (9 cases, 28%), aspiration (7 cases, 23%), pulmonary infection (5 cases, 16%), pulmonary emphysema (5 cases, 16%).

In the control group, a remarkable lower number of the six most common findings detected by CT in the heroin and methadone group was found in pulmonary edema (28%), distended urinary bladder (14%), pulmonary infection (3%), and in pulmonary emphysema (3%). Findings of aspiration (21%) and cerebral edema (28%) were almost similar to the heroin/methadone group.

The combination of lung and brain edema with a full bladder was seen in the heroin/methadone group in six cases (19%), whereas this combination was found in the control group in only one case (3%).

This study demonstrates characteristic findings of postmortem whole body MSCT in cases of heroin and methadone abusers. Furthermore, the combination of MSCT findings of a distended urinary bladder, edema of brain and lungs appears to be more specific than originally assumed, however much not very sensitive. Their combination should raise suspicion of intoxication even though some of the cases contained, but were not judged to have died of, heroin and/or methadone. While this appears to be well known in forensic pathology, new synthetic drugs that might not show up on toxicology screening tests in conjunction with restricted resources for routine toxicology might put an additional burden on forensic pathologists to argue case angles of possible poisoning based on evidence.

These preliminary results are promising regarding the value of postmortem imaging in deceased persons associated with heroin or methadone consumption. A future step is to evaluate the number and types of findings in larger studies.

Forensic Radiology, Computed Tomography, Drug Abuse

G66 Comparative Evaluation of Radiolucent Projectile Components by Radiographs and Computed Tomography

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After attending this presentation, attendees will understand the necessity and limitations of forensic imaging in autopsies of suspected gunshot victims. Attendees will gain an understanding of the

importance of recovery of traditionally radiolucent projectile components at autopsy and of the challenges presented in this pursuit by the use of postmortem radiography only as opposed to radiography supplemented with Computed Tomography (CT).

This presentation will impact the forensic science community by strengthening the validity and necessity of postmortem CT scanning for gunshot-related fatalities. These findings also have applications clinically, as missed radiolucent projectile components left in the body during surgery can lead to infection.

Projectile components that are traditionally radiolucent are important in determining weapon and projectile type and caliber, but these components are often not visualized on postmortem radiographs. It was hypothesized that these components would be better visualized by evaluation of CT scans compared to the forensic pathology practice standard of X-ray.

Thirty-two cartridges with projectiles that possessed a radiolucent component were both dismantled using an interia bullet puller and fired, either into a water tank or down an enclosed range, and the radiolucent components were recovered. These cartridges were comprised of a variety of centerfire handgun, shotgun, and rifle rounds. The components were embedded in disrupted zones of blocks of ballistic gelatin. Nine true negative areas of disruption were also created. The blocks were subjected to X-ray and CT scanning. The CT images were helically acquired and reconstructed in axial, coronal, and sagittal views at slice thicknesses of 1.0mm x 0.5mm, 3mm x 3mm, and 0.8mm x 0.4mm. The X-ray and CT images were evaluated by three blindfolded, board-certified radiologists (two with training in forensic radiology) for the presence/absence of projectile components. If a projectile component was present, the radiologists further described their observations.

Projectiles were broken into several categories for analysis: fired vs. unfired, X-ray vs. CT scan, and material type. The material type category was further delineated as plastics, metals, cardboard/fiber, and miscellaneous, which included rubber, styrofoam, paper, fabric, and nylon. In all instances concerning observers one and three, visualization of projectiles/projectile components was equal or better on CT scans when compared to X-ray. This was true for observer two in only the cases of unfired projectiles (0% difference), metals (0% difference), and miscellaneous projectiles (7.7% difference).

An unpaired one-tailed Student t-test ($p<0.05$) was performed to compare total numbers of projectiles/pojectile components identified correctly on X-ray versus CT scan for each observer. The null hypothesis, that there was no difference in observation of projectiles/projectile components on CT scan vs. X-ray, was rejected ($t_{crit} = 2.92$, $t = 17.022$).

Though inter-rater kappa values between the three observers were low in most instances, the percentage of projectiles identified of any material type was high. The kappa values for observers one and two were 0.075 overall, -0.019 for CT, and 0.721 for X-ray. For observers one and three, kappa values were 0.255 over all, 0.333 for CT, and 0.139 for X-ray. For observers two and three, kappa values were 0.841 overall, -0.037 for CT, and 0.104 for X-ray. Observer one identified 88.8% of projectiles correctly, with 78.6% on X-ray and 99% on CT, a 20.4% increase. Observer two identified 84.7% of projectiles correctly, with 89.8% on X-ray and 79.6% on CT, a 10.2% decrease. Observer three identified 96.9% of projectiles correctly, with 95.9% correct on X-ray and 98% correct on CT, a 2.1% increase.

Of the true negative zones created 70% were described by the radiologists. Of these, 55% were correctly identified as true negatives and the remainder as false positves. False positives were identified in 98 cases on X-ray (obvserver 1:2; 2:64; 3:32), and 87 false positives were identified on CT (observer 1:0; 2:80; 3:7).

Traditionally radiolucent projectiles/projectile components embedded in ballistic gelatin blocks were significantly better visualized with CT scans than X-ray. Though concordance between radiologists was low, the total percentages of projectiles identified by each radiologist in each modality were high. The disparities between radiologists' identification of false postives can be attributed to individual skill level and familiarity with projectiles. As gelatin blocks have densities similar to human soft tissues, CT would likely be a better modality to find these projectile components in people with fatal and non-fatal gunshot wounds.

Forensic Pathology, Wound Ballistics, Forensic Radiology

G67 Determination of Postmortem Interval Using Non-Invasive Magnetic Resonance Imaging Measurement of the Apparent Diffusion Coefficient and 1-Dimensional Spectroscopy

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After attending this presentation, attendees will: (1) understand the application of non-invasive magnetic resonance Imaging; (2) become familiarized with Magnetic Resonance Spectroscopic Imaging (MRSI); and, (3) understand what this advanced technology entails and how this technology will aid in the determination of postmortem interval.

This presentation will impact the forensic science community by showing how this rarely used technology in forensic investigation can potentially help address important forensic questions such as postmortem interval.

Postmortem Magnetic Resonance Imaging (MRI) with Diffusion Weighted Imaging (DWI) to produce an Apparent Diffusion Coefficient (ADC) map is being refined as a method for determining Postmortem Interval (PMI) over the 2-14 day range. DWI can be used to monitor decomposition, because tissue breakdown results in increased diffusivity of tissue water corresponding to an increased ADC value. It was hypothesized that the addition of Magnetic Resonance Spectroscopic Imaging (MRSI) will enable the decomposition mechanism (autolysis only vs. putrefaction) to be identified. Spectroscopy enables the identification of specific chemical species, in this case metabolites resulting from decomposition. Currently, these methods are being tested using mammal models with known PMI. In the future, the combination of ADC mapping and MRSI is expected to provide a non-invasive method of determining PMI in specimens or decedents whose time of death is not known.

Examination using MRI with ADC mapping is performed on a 1.5 T MRI scanner on *in situ* mammal brains over the period of two weeks. The mammal specimens decompose at room temperature. Five specimens have been imaged thus far. At each postmortem time point, six echo planar images are acquired with an increase in the diffusion weighting with b-values of 0, 500, 1,000, 1,500, 2,000 and 2,500 s/mm^2. B-values are a measure of the strength of the diffusion sensitizing gradients used during DWI. The individual pixel elements are plotted as a function of the b-value. The slope depicts the ADC in units of mm^2/s. All the ADC values from the six echo planar acquisitions are calculated together to create an ADC map. Regions of Interest (ROI) are obtained from common tissues within the brain; i.e., gray matter and white matter. The ADC values obtained from these ROI are plotted versus PMI, enabling the creation of standardized curves quantifying the continuous change in the ADC of the tissue with increasing PMI. Volume-selective 1D MRSI is currently being added to the above protocol to identify and quantify specific metabolites in the decomposing tissue.

Preliminary data from five specimens reflects two distinct ADC vs. PMI curves that were hypothesized and corresponded to decomposition by different mechanisms: autolysis and putrefaction. The autolyzed tissue follows a steady incremental incline in the ADC curve and levels off with a lower numeric ADC value ≈ 22 mm^2/s. The lower numeric value signifies the tissue has less fluid movement and is more intact. The putrefied tissue demonstrates a rapid incline in the

ADC curve and levels off at a higher numeric ADC value ≈ 37 mm^2/s. Putrefied tissue exhibits more diffusion, or water movement, due to more complete cellular breakdown of the tissue. Thus far, the five specimens studied follow one of the two curves described above, presumably based on the nature of decomposition. The identification of the two curves as "putrefaction" and "autolysis" is based on standard MR anatomic imaging of each specimen, which shows that the tissues that yield a higher ADC value also demonstrate more significant gross structural changes and more gas inclusions, highly suggestive of bacterial putrefaction.

To confirm these results, further ADC studies of mammal models are underway to create statistically significant and reproducible results. To elucidate the nature of the two different ADC vs. PMI curves observed, MRSI, a non-invasive method to chemically analyze tissues, is being added to the protocol to reveal if the tissue is undergoing bacterial decomposition or autolysis. The analysis of the spectral data obtained will focus on identifying metabolites that differentiate biotic from abiotic decomposition. The outcome of the spectral analysis is therefore expected to indicate which of the two observed ADC curves the tissue should follow, which will allow accurate interpretation of ADC values obtained from tissues with unknown PMI in future studies.

Forensic Radiology, Magnetic Resonance, Postmortem Interval

G68 Pseudo Searing of the Skin Around Contact Gunshot Wounds

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The goals of this presentation are to challenge the assumption that blackened skin around contact gunshot wounds is due to heat and to offer an alternative explanation for the cause of the skin color change.

This presentation will impact the forensic science community by presenting new data to challenge the longstanding belief that the black area of skin around a contact gunshot wound is due to soot forced into the skin rather than burning of the skin (or searing) from heat.

Searing of the skin by hot gases from contact gunshot wounds has been long documented in the forensic pathology literature; however, it has not been reliably shown that hair on and around the skin of the wound is burned by the hot gases of combustion. If the heat of the combustion is high enough to burn the skin, then it should affect the hair in and around the skin as well. Burning requires enough heat energy be transferred to the tissue to cause injury. The muzzle flash and the hot gases expelled from the end of the barrel are very brief in duration, even though the temperature has been measured at 1700K to 2000K (1400°C to 1700°C). The effect on the hair is minimal as the heat energy is small. The hypothesis is that hair burns much more easily than skin. Simple flame tests were performed on hair-bearing skin and the effect of the heat and flame documented. The hair burned and was consumed without evidence of any visible damage to the underlying skin.

A retrospective review of color photographs of 270 cases of contact gunshot wounds to the head and chest that occurred from January 2010 to July 2012 was conducted to assess the condition of the hair around the wound. The finding of unaffected hair around the wounds suggests that the black area of skin around a contact gunshot wound is not due to heat applied to the skin by gases. The review cases showed a wide variety of calibers and power variation. The microscopic features described in the literature can potentially also be explained by the soot being pushed at high pressure into the tissues around the wound that have already been abraded by the passing of the projectile. The features are therefore a kinetic energy effect rather than a heat or burn effect. The disrupted appearance of the cells with soot ingrained into the area gives the appearance of searing. Microscopic sections of tests fired through donated cadaver scalp at different ranges from contact to distant using 9×19mm Parabellum ammunition will be presented showing the features of each range.

In 127 cases, the photographs reveal that the hair was not burned. The remaining cases did not have adequate photographs to assess the status of the hair, or the wound was in an area of the body with no surrounding hair.

The longstanding belief that skin is burned or seared during a contact gunshot holds no merit. The photographs from 127 cases reveal that the hair in and around the wound remains intact and, since hair is more susceptible to heat damage than skin, allows the previously documented theory of searing to be reasonably challenged.

Contact Gunshot, Searing, Hair

G69 Retinal Hemorrhages Associated With Partial Submersion (Near Drowning)

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After attending this presentation, attendees will learn that retinal hemorrhages can occur in cases of partial submersion (near drowning) with cardiopulmonary resuscitation and should not be considered specific for abusive head trauma in young children.

This presentation will impact the forensic science community by illustrating that retinal hemorrhages in young children must be interpreted with caution, particularly in the setting of extended cardiopulmonary resuscitation.

Aggressive, prolonged Cardiopulmonary Resuscitation (CPR) often occurs in partial submersion (near drowning) in young children. A literature review did not reveal any reports of Retinal Hemorrhages (RHs) associated with partial submersion or drowning, and there have been only a few studies looking at RHs associated with resuscitation in children. Two cases of RHs in young children who died following partial submission (near drowning) were presented. In both cases the decedents received cardiopulmonary resuscitation and advanced life support.

Case 1: A 7-year-old male experienced a partial submersion (near drowning) incident at a hotel swimming pool. He was witnessed flailing, choking, and struggling to stay above water. His mother and another adult helped him to the side of the pool. Emergency Medical Services (EMS) arrived and reported that he was breathing spontaneously and was in normal sinus rhythm. During transport to the Emergency Department (ED), he became bradycardic and then asystolic. CPR was started and continued for 25 minutes after arrival at the ED before he recovered spontaneous circulation. Cranial Computed Tomography (CT) showed profound global hypoxic-ischemic injury with cerebral edema. He was in the hospital for two days during which time his neurological status continued to decline and a repeat CT showed worsening cerebral edema with transtentorial and tonsillar herniation. Clinical brain death was pronounced. No clinical fundal examination was documented in the medical record. At autopsy, Postmortem Monocular Indirect Ophthalmoscopy (PMIO) revealed 50-100 bilateral flame-shaped and dot-blot retinal hemorrhages extending over the posterior poles and focally abutting the ora serrata. No optic nerve sheath hemorrhages were identified. Neuropathological examination confirmed severe, diffuse hypoxic-ischemic brain injury with cerebral edema and cerebellar tonsillar herniation.

Case 2: A 6-year-old male was found submerged in a swimming pool at a campground. He was estimated to have been submerged for approximately ten minutes. Bystander CPR was performed at the scene and EMS was alerted. EMS reported that the decedent was cold, unresponsive, and asystolic upon arrival. CPR was continued and Pediatric Advanced Life Support (PALS) was initiated en route to the nearest hospital. Aggressive resuscitation was continued in the ED until a circulating rhythm was established. He was admitted to the

Pediatric Intensive Care Unit (PICU) with a preliminary diagnosis of anoxic brain injury. His temperature was normalized, but he remained comatose throughout this course in the PICU with a Glasgow Coma Score of three. His pupils were fixed, dilated, and nonreactive. He had no cough, corneal, gag, or deep tendon reflexes. A head CT showed diffuse edema with global hypoxic/ischemic injury, and a nuclear medicine brain scan showed no intracranial blood flow confirming brain death. No clinical fundal examination was documented in the medical record. At autopsy, PMIO revealed four flame-shaped retinal hemorrhages in the right eye, and no abnormalities in the left eye. No optic nerve sheath hemorrhages were identified. Other findings included acute bronchocentric pneumonia, pulmonary edema, and diffuse hypoxic-ischemic brain injury with cerebral edema and tonsillar herniation.

These two cases further expand the spectrum of retinal hemorrhages in children with cerebral edema. RHs in children have typically been viewed as a hallmark for abusive head trauma, but routine postmortem ocular examination has revealed increasing numbers of cases with RHs not associated with AHT. RHs have not been previously associated with partial submersion (near-drowning). The RHs in these two cases are most likely attributable to resuscitation efforts and ischemia-reperfusion injury, but further investigation is required to determine the cause of retinal hemorrhages following partial submersion (near-drowning) with resuscitation.

Retinal Hemorrhages, Near Drowning, Resuscitation

G70 Perimacular Retinal Folds Associated With Fatal Intra-Cranial Injuries in Adults From Same Height and Near Same Height Falls

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After attending this presentation, attendees will learn that Perimacular Retinal Folds (PRFs) can occur in adults who have fatal traumatic brain injuries from same height or near same height falls.

This presentation will impact the forensic science community by emphasizing the utility of postmortem monocular indirect ophthalmoscopy and highlighting the need for caution in attributing the mechanism of PRF formation to vitreo-retinal traction occurring during cycles of cranial and ocular acceleration-deceleration.

Perimacular retinal folds are considered virtually pathognomonic for Shaken Baby Syndrome (abusive head trauma). However, PRFs have not only been identified in accidental infant head trauma, but have been identified in adults with Terson syndrome. The proposed mechanism of the intra-ocular hemorrhage is a rapid increase in intracranial pressure. Four decedents presenting with perimacular retinal folds at autopsy associated with fatal intracranial injuries are described. They all had blunt force head trauma secondary to same height/near same height falls.

Case 1: A 55-year-old woman was found dead lying at the bottom of a staircase. An area of blood was on the staircase. She reportedly had consumed alcohol the night prior to her death.

Autopsy findings included a 130gm left acute subdural hematoma (SDH) with flattening of the left cerebral hemisphere and a left-to-right shift with transfalcine hermiation, a 4th rib fracture, and a right temporal scalp hematoma. She had bilateral optic nerve sheath nerve hemorrhages (left greater than right) and an 8 x 5mm, well-demarcated, premacular pre-retinal hemorrhage circumscribed by a PRF.

Case 2: A 54-year-old man with a past medical history of alcoholism and esophageal cancer fell off a porch while drinking. Family witnessed the fall and called emergency services. On arrival at the hospital he was comatose. Cranial imaging showed florid cerebral edema with generalized ischemic injury and brain stem herniation. Four days later he was pronounced dead. No clinical fundal examination was documented.

Autopsy findings included facial abrasions, hemorrhage involving the temporalis muscles, bilateral SDH, bilateral subarachnoid hemorrhage, marked cerebral edema, and microscopic findings consistent with hypoxic ischemic brain injury. Postmortem ocular examination revealed bilateral optic nerve sheath hemorrhages, multiple retinal RHs in the right globe, and peripapillary hemorrhage associated with subretinal hemorrhage in the left globe.

Case 3: A 37-year-old man was transported to the emergency room. He had been in a physical altercation and fell striking the back of his head. A cranial CT revealed a SDH with significant rightsided cerebral swelling. His brain injury was considered non-survivable and life support was discontinued. No clinical fundal examination was documented in the medical record.

Autopsy findings included trauma to the left inferior occipital region with complex basilar skull fractures, bilateral frontal SDHs, subarachnoid hemorrhages, transtentorial herniation, and intraparenchymal hemorrhages in the cortex and brainstem. Bilateral optic nerve sheath hemorrhages were identified, PRFs, multiple retinal hemorrhages (including premacular hemorrhages in both globes), rupture of hemorrhages into subhyaloid space and vitreous, shearing of outer segments of photoreceptors by dissecting retinal hemorrhages, and subretinal hemorrhage with retinal detachment.

Case 4: A 60-year-old woman had a past medical history of repeated altered mental status and polysubstance abuse. She was found unresponsive at home and transferred to the nearest medical center. Cranial computed tomography revealed bilateral SDHs. No clinical fundal examination was documented in the medical record.

Autopsy findings included a laceration of the posterior scalp, bilateral SDHs, cerebral edema, right parafalcine and uncal herniation, midbrain/pons Duret hemorrhages, bilateral optic nerve sheath hemorrhages, scattered RHs over the right posterior fundus, and a premacular hemorrhagic cyst with a PRF in the left eye.

These four adults with retinal hemorrhages and PRFs died from traumatic brain injuries due to same height/near same height falls and expands the number of conditions associated PRFs. Three of four decedents died following hospitalization; however, no clinical fundal examinations were documented in their medical records. The hemorrhagic retinopathy and PRFs were identified by postmortem monocular indirect ophthalmoscopy emphasizing the need for uniform and consistent ocular examination of decedents with fatal traumatic brain injuries.

Retinal Folds, Accident, Brain Injury

G71 Death Due to Excited Delirium Associated With Synthetic Marijuana Use: A Review of Two Recent Cases

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After attending this presentation, attendees will understand the toxicity of synthetic cannabinoids, the literature regarding associated deaths, and a case series of the two deaths of excited delirium resulting from synthetic marijuana use.

The presentation will impact the forensic science community by describing excited delirium due to synthetic cannabinoids and elucidate the need for further regulation of these substances.

Subjects in excited delirium typically present with aggressive, agitated, bizarre, and combative behavior and display invulnerability to pain, hyperthermia, diaphoresis, tachycardia, a tendency to be under-clothed for the environment, and a propensity toward water,

lights, or glass. In fatal cases, the subject frequently has a history of a struggle, restraint or sedation, sudden cardiac arrest and death while in custody or at the hospital, positive drug screen, and no other anatomic cause of death identified at autopsy. The majority of cases involve stimulant abuse, predominantly cocaine, but also methamphetamine, Lysergic Acid Diethylamide (LSD), Phencyclidine (PCP), etc. Less commonly, the subject may have a psychiatric history with a sudden discontinuation of antipsychotic medications.

The use of synthetic marijuana is on the rise, and there have been reported cases of fatal outcome associated with consumption of these compounds. Synthetic marijuana is popular among young people and is the second-most-used drug by high school seniors nationwide. Synthetic marijuana is marketed as a mixture of traditionally used medicinal "herbal incense" with mild cannabis-effect and sold under many names, such as K2, Spice, legal phunk, etc. Synthetic cannabis is a mixture of plant ingredients blended with synthetic cannabinoids such as cannabicyclohexanol, JWH-210, JWH-018, JWH-073, HU-210, JWH 250, RCS-4, RCS-8, etc. Case reports have described acute psychosis, convulsions and seizures, and myocardial infarction occurring after synthetic cannabinoid use. There are reported cases of sudden death following synthetic cannabinoid and anecdotal reports of agitated and bizarre behavior related to use, however, the literature is lacking in reported cases of excited delirium fatality secondary to synthetic cannabinoid consumption.

Two recent fatal cases of excited delirium associated with the use of synthetic marijuana were reported. A similar series of events occurred for both individuals, in which both subjects were acting in a bizarre and agitated manner prompting a struggle between the subject and police, resulting in restraint of the subject's arms in handcuffs behind the back and restraint at the ankles. In both cases, the subjects were witnessed to become unresponsive while in custody, arrived at emergency medical care in cardiac arrest, and had hypoxic ischemic encephalopathy with cerebral edema and cerebellar tonsilar herniation at autopsy. A history of recent synthetic cannabinoid use was related for both subjects, and both subjects had positive hospital drug screens for cannabinoids during their hospital course. Routine postmortem toxicological screening on urine and whole blood were negative. Additional testing for synthetic cannabinoids was performed by NMS Labs (Willowgrove, PA) using a liquid-liquid extraction technique and liquid chromatography-positive ion electrospray ionization tandem mass spectrometry**.** Postmortem testing of the hospital admission blood of the first subject was positive for the synthetic cannabinoid JWH-210. Test results for additional illicit drugs, including bath salts were negative in both cases.

Two cases of fatal Excited Delirium (ED) due to synthetic cannabinoid use are described. The use of these drugs is on the rise, especially among younger individuals, and the presence of these drugs should be suspected in cases of ED when other drugs known to cause ED, such as cocaine, phencyclidine, and bath salts are negative.

Spice, Synthetic Marijuana, Excited Delirium

G72 Ruptured Intracerebral Arteriovenous Malformations, Hypertensive Cardiovascular Disease, and Acute Hypertensive Effects of Stimulants: Is There a Direct Link?

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The goal of this presentation is to provide an understanding of the relationship between hypertensive cardiovascular disease and rupture of intracerebral Arteriovenous Malformations (AVMs).

This presentation will impact the forensic science community by improving the understanding of the impact of hypertensive cardiovascular disease on the natural history of intracerebral AVMs. The relationship of AVM rupture with the acute hypertensive effects of drugs will also be explored.

Currently available data regarding natural history of AVMs is conflicting; clinical studies have been limited by small sample size, young age of patients (prior to peak incidence of hypertension), and lack of long term follow-up.[1-5] Accordingly, some have concluded that hypertension has no impact on rupture of AVMs, while other sources include hypertension as a major risk factor for AVM rupture.[4-6] Therefore, the risk of hypertensive cardiovascular disease on intracerebral AVMs is presently unclear. Whether rupture of an AVM may be precipitated by or associated with an acute hypertensive episode caused by substance abuse (e.g., cocaine, methamphetamine, and phencyclidine) has direct relevance for manner of death.

A retrospective cohort study was designed in which all autopsy case files between January 2005 and July 2012 were reviewed to identify adult decedents (age >18 years) in which an AVM was listed as a cause of death. Cases were excluded if the decedent had a remote history of neurosurgical intervention. Other types of intracranial fistulas (i.e., dural arteriovenous malformations) and nonfistulous malformations (i.e., cavernous hemangiomas) were excluded. One case of unruptured AVM was included.

A total of 11 cases were identified that met study criteria. Relevant biographical and historical information was collected. Gross autopsy and histopathological data were collected on each decedent to assess for the presence or absence of cardiomegaly, left ventricular hypertrophy, end organ (kidney) damage, and extent of involvement and chronicity of the AVM. Heart weights were compared to expected heart weights determined from a model described by Gaitskell *et al*. in 2011.[7]

Seven of the 11 cases (64%) were male and 4/11 (36%) were female, with an average age of 44 years (range 29 – 58 years). Average body weight was 177 pounds (range 118 – 248 pounds) and average height was 66.5 inches (range 62 – 70 inches). The average body mass index, characterized as overweight, was 28.2kg/m^2 (range 17.9 – 39.1kg/m^2). The cases comprised of mostly (91%) ruptured AVMs with associated hemorrhage: Subarachnoid (SAH) 8/10 (80%); subdural (SDH) 2/10 (20%), and intraparenchymal 5/10 (50%).

Autopsy heart weights averaged 422g (range 250 – 690g) with an average left ventricular wall thickness of 1.5 cm (range 1.0 – -2.0cm). Four of ten (40%) decedents with ruptured AVMs had both cardiomegaly and left ventricular hypertrophy (left ventricular thickness >1.4cm). Five of the ten (50%) ruptured cases had Left Ventricular Hypertrophy (LVH) and 3/4 (75%) of the cases with cardiomegaly and LVH also had evidence of hypertensive kidney disease (nephrosclerosis and arteriolosclerosis). Ultimately, six out of ten (60%) of the cases with ruptured AVMs had evidence of hypertensive disease as evidenced by one of the following: LVH, cardiomegaly, or hypertensive kidney disease. A clinical history of hypertension was identified in two of 11 (36%) of the cases (each confirmed by histopathology); one case was an unruptured AVM and one was a ruptured AVM. Finally, two of the ten (20%) decedents with ruptured AVMs also had acute cocaine toxicities; one had both hypertensive heart and kidney disease, the other had histopathologic evidence of hypertension. Each was classified as an accident.

Ultimately, it cannot be concluded from these data that hypertensive cardiovascular disease is a direct risk factor for rupture of intracerebral AVMs. However, the low percentage of hypertension in decedents with ruptured AVMs suggests the association is weak at best. This data would support a cautious approach when assigning manner of death to decedents who died from a ruptured AVM and also had stimulants detected during autopsy. Furthermore, whether to include underlying hypertension as a contributory cause of death is not clear. A major limitation of this study is the low number of study subjects.

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Arteriovenous, Malformation (AVM), Hypertension

G73 Neonaticide: A Retrospective Analysis of Characteristics and Trends of 55 Cases

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After attending this presentation, attendees will learn: (1) common demographics of neonaticide victims, including circumstances surrounding the pregnancy, crime scene characteristics, method of discovery, and cause of death; (2) common characteristics of neonaticide offenders; and, (3) diagnostic tests and difficulties in neonaticide investigations.

This presentation will impact the forensic science community by increasing the awareness among criminal justice professionals and forensic pathologists of the potential investigative challenges in neonaticide cases and will provide recommended investigative techniques.

Neonaticide is frequently defined as the killing of a newborn, most commonly by the biological mother, within 24 hours of birth. While not uncommon, this crime is difficult for pathologists, investigators, and families alike to understand. This FBI retrospective study analyzes the largest known collection of neonaticide cases from 20 states between the years 1992 to 2009 in an effort to better understand this crime and how to recognize it at the time of autopsy. Fifty-four female offenders with 55 related infant deaths (one offender killed two infants in separate incidents) were identified through the FBI's internal databases, law enforcement partners and public-source information. The perpetrators' average age was 21.7 years. Thirty-three percent had other living biological children, while 43% had previously been pregnant (although not all resulted in live births). Most of the perpetrators (96%) were hiding the pregnancy from family members. While all offenders knew they were pregnant and confirmed this to a family member or friend, only 5% of these women had the pregnancy confirmed by a medical doctor. Regarding victims, male victims (55%) were slightly more common than female victims (45%). Caucasians (49%) made up the majority of victims, with Hispanic (18%), Bi-racial (15%), African American (9%), Native American (6%), and Asian (4%) victims being less common. Fifty-eight percent of victims were discovered in an indoor location, the offender's residence being the most common place of discovery (53%). Victims discovered at an outdoor location (33%) were usually found outside the perpetrator's residence in a trashcan, the backyard, or on the roof. Most commonly (38% of cases), the victims were discovered once police were dispatched to the home after the mother sought medical attention, was found to have recently given birth, and medical personnel contacted authorities. The time interval between birth and discovery was less than 24 hours in 77% of the cases where the time interval was recorded (13 cases). In 75% of cases, no decomposition of the victim was identified. Five percent had mild decomposition, 5% showed moderate decomposition, and 15% had severe decomposition. While only in 87% of cases did a forensic pathologist specifically list "live birth" on the autopsy report, all cases were presumed to be live births since the deaths were ruled as homicides. Most pathologists used a combination of methods to determine live birth (89% of cases), with the combination of the hydrostatic "float" test on the lungs, microscopic evaluation of the lungs and other tissues, and confirmation of air in the lungs and gastrointestinal tract via gross examination or X-ray being the most common combination of methods (27.5%). Forty-five percent of cases relied on the history to help determine live birth, with 16% of cases relying solely on history, as Cause of Death (COD) could not be determined. The COD was determined in 84% of cases, with asphyxia deaths (80%) being the most common cause. Of these, 62% were due to suffocation, 30% were due to drowning, and 8% were due to strangulation. Of those deaths where a single COD was identified, 13% were due to sharp force injuries, 7% were blunt force injuries and 7% were due to exposure. In 27% of cases, the pathologist ruled the COD as multifactorial, with suffocation and exposure being the most common combination (73%). In neonaticide, as in many other forensic pediatric deaths such as SUID and drowning, the COD is made on the totality of the circumstances. The pathologist must consider the historical information, scene investigation, and pathologic findings when rendering an opinion regarding cause and manner of death.

Neonaticide, Child Homicide, Pathology

G74 Jay Dix Memorial Bonus Day

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After attending this presentation, attendees will learn how and why deaths related to multitude of topics 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 receiving a comprehensive review of what causes and contributes to different types of deaths. Attendees will be able to systematically evaluate deaths in which the previously specified topics that may have played a role in their daily practices.

A proper medicolegal death investigation is a multidisciplinary process that often involves non-medical personnel as well as medical professionals. This annual series of discussions is intended to provide the non-forensic pathologist forensic scientist a comprehensive basic review of selected topics in forensic pathology in order to increase familiarity and understanding and enhance inter-discipline communication.

This year's presentations will discuss the investigation of infant deaths and deaths related to electrocution, asphyxia, sports and recreation, and suicide.

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 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 presentation will provide a comprehensive review of these issues.

Human life requires the uptake and utilization of oxygen along with the release of metabolic waste. Failure of these processes leads to asphyxia. There are numerous entities—mechanical and chemical—that can cause asphyxia through a variety of mechanisms, present in a wide range of scenarios and that can be associated with a broad range of physical findings. Proper evaluation of these deaths requires knowledge of the various entities that can cause asphyxia, mechanisms through which these agents affect physiological function, scenarios under which these deaths occur and factors that contribute to these deaths. This presentation will comprehensively discuss the investigation of death in which asphyxia may have played a role.

There are multiple causes, mechanisms, and contributory factors that can play a role in deaths that are temporally related to participating in and, occasionally, while being a spectator at sporting or other recreational activities. Understanding these deaths requires understanding of the physical requirements to perform particular activities, susceptibility of particular diseases to stresses associated with particular activities, effects of various chemical and/or biological agents that may be taken to enhance performance and physical injuries associated with particular recreational activities. This presentation will provide a comprehensive review of these issues in the context of investigating deaths that occur in relation to sports/recreational events. Understanding factors that are involved in deaths occurring in these circumstances also helps in instituting appropriate safety measures to protect participants and spectators.

The intentional termination of one's own life, suicide, can be accomplished in many ways, some overt and others covert. The accurate recognition of suicide has important implications for the decedent's survivors, estate, and others such as law enforcement. Unlike most other manner of death determinations, an assertive determination of the decedent's intent is fundamental in certifying a death as suicide. In addition to accurately determining the cause and manner of death, the proper investigation of a suicidal death may offer insight into the motivation for the death and provide information in the development and implementation of preventive strategies. The investigation and interpretation of findings in suicidal deaths will be comprehensively discussed.

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. The investigation and interpretation of findings in sudden unexpected deaths involving infants will be discussed.

Sudden Death, Death Investigation, Forensic Pathology

G75 Unusual Suicides Utilizing Chain Saws — Case Reports and Literature Review

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After attending this presentation, attendees will understand what can be observed in an unconventional suicide such as one committed with a chain saw.

This presentation will impact the forensic science community by better preparing those conducting death investigations for the analysis of unconventional death scenes and autopsy results to help identify the manner of death in these unusual cases.

Purposeful application of a chainsaw to the human form is a rare event; however, it has been reported in the literature more frequently in the past several years in cases of self-mutilation, suicide attempts, and postmortem dismemberments. In this presentation, the only two cases of suicide by chainsaw that have occurred in Los Angeles County will be reviewed. The findings of the investigation including the initial death scene examination, autopsy results, and subsequent analysis of tool marks in bones will be discussed. The mechanisms of suicide utilizing a chainsaw in these case reports will be examined and compared to other cases reported in the literature.

Case 1: A 49-year-old male was found dead on the floor of a motel room. He had been a resident of a board and care facility for the mentally impaired for over six years, but was not known to be under a physician's care for psychiatric issues at the time of his death. He had checked into the motel the day before for a one-night stay. He was lying supine between the foot of the bed and a dresser and the electric chainsaw was laying on the bed. Nearby on a table were the decedent's driver's license, suicide note, receipt for the chain saw dated the day prior and other personal items laid out in an orderly fashion.

Autopsy findings: A single wound approximately 2¼ inches deep passing through the cervical vertebrae but not injuring the spinal cord extended from just posterior to the right ear inferiorly across the neck producing a wound approximately 7 inches long. The atlas (C1) vertebra revealed injuries compatible with the use of a large width cutting-edge instrument such as a chain saw, reinforced by the presence of chatter across the occipital bone.

Case 2: A 47-year-old female was found dead in her bedroom. She was considered disabled due to a brain injury but was not known to be under a physician's care for psychiatric issues. She was lying supine with her head at the foot of the bed. The electric chainsaw was present on the bed with the blade resting across the decedent's neck and her right hand still in the handle. The sheets she had covered the dressers with and the comforter on the bed were covered with blood spatter. A suicide note was taped to a wall of the bedroom.

Autopsy findings: There were two deep incised wounds, one of the anterior neck and one on the left shoulder. The wound of the neck measured approximately 6 inches long and extended from just inferior to the right submandibular angle to the left side of the neck and continued through to the posterior surface of the neck just left of the posterior cervical line. The wound to the left shoulder, which appeared to originate in the left shoulder articulation and terminate in the left clavicle, was approximately 2 inches in length. A study of the neck organs, cervical vertebrae, and clavicle revealed injuries compatible with the use of a large width cutting-edge instrument, such as a chain saw.

Commonalities among the two cases included: no history of suicide attempts or suicidal ideations, no defensive or hesitation wounds, and toxicological results indicating no drugs of abuse present at the time of death.

Suicides by chainsaw are rare but reported in the literature. There can be discrepancies between what is found at the death scene and autopsy of these unusual suicides and what is expected to be found in a conventional suicide. These cases were compared to the

literature in consideration of the commonalities between the two cases and the differences that make each of these cases unique in comparison to the current literature.

Chainsaw, Suicide, Forensic Pathology

G76 Accidental Injury Caused by a Handheld Circular Saw: An Unusual Industrial Accident

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After this presentation, the participants will understand how this fatal accident occurred as a result of improper use of a handheld circular saw, as well as how the scene investigation gave investigators some insights on how a tool designed to prevent fatal injuries was involved in an accident that caused serious lesions to the user.

This presentation will impact the forensic science community by showing how multidisciplinary investigations, with the participation of the tool manufacturer, occupational health and industrial safety staff, and medical examiners, were instrumental in reconstructing the chain of events in a unusual industrial accident.

Presented here is a case of death resulting from an unusual industrial accident caused by the laceration of internal thoracic and abdominal organs. The accident occurred when a carpenter fell on a handheld circular saw while he was cutting wood. This report analyzes the scene, the chain of events, and the pathogenetic mechanisms that caused the death of an individual who was in the process of performing an unauthorized activity. The forensic aspects of the case will be discussed. Upon reviewing the existing literature, a large number of cases where various types of saws were used in suicide attempts are reported, but no reports were found on this kind of accidental death due to this type of tool. Under normal conditions, the handheld saw is used by an operator who directs the sharp edge of the saw to a fixed wood fragment located below the saw. The cutting action should not cause any problems because the tool has a protection mechanism (cover). In the case reported, the operator did the opposite. He attached the saw to a table with the cutting edge facing upwards, removed the cover, and put the wood on the cutting edge of the saw. He tripped on the wires and fell on the saw. The operator suffered serious injuries that affected his vital structures of the chest and abdomen, which caused his death due to massive bleeding of the right lung, the heart, and the abdomen. When the scene was processed, it was documented how the saw was improperly attached to the table and how the industrial accident happened. Other saws in the factory were checked in order to determine the cause of the injuries. A case of this type of accidental death has never been reported in the literature.

This case will make an impression on the international forensic community, since it will show how multidisciplinary investigations, with the participation of the tool manufacturer, occupational health and industrial safety staff, and medical examiners, all of whom were instrumental in reconstructing the chain of events. The multidisciplinary team was able to determine the factors involved in this unusual industrial accident. At first it was difficult to determine the manner of death. There was disagreement between the authorities that processed the scene and the medical examiners. The tool involved in this fatality was examined by an expert who found that the expectations of the manufacturer were correct and concluded that the tool had been misused. The medical and psychiatric records of the deceased were reviewed for some kind of psychiatric disorder that could explain the fatality. Since occasionally labor accidents involve the use of drugs of abuse, a toxicology screening of the vital fluids was carried, with negative results.

A case will be presented where an unusual industrial accident with a handheld circular saw was examined. This is an example of the practical application of multidisciplinary investigations and cooperation in order to solve unusual deaths. Forensic investigators must become familiar with tools that are increasingly involved in suicide attempts and fatal accidents.

Industrial Accident, Fatal, Investigation

G77 Circular Saw: No Enigma?

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The goal of this presentation is to present a fatal case with a sharp injury on the neck due to a rotating saw blade in a man with occupational experience with power saws. A detailed crime scene investigation, and a complete postmortem examination established the cause of death and excluded homicide and suicide.

This presentation will impact the forensic science community by emphasizing the importance of a careful crime scene investigation in order to differ between accidental, suicidal, or homicidal events when investigating injuries of the neck in cases involving a circular saw.

Fatal cases involving the use of power saws (band saws, circular saws, or chain saws) are rarely reported in literature. The injuries are most likely to be to the front of the trunk, head, or neck, but may be in any part of the body where there is a blood vessel large enough to give rise to rapidly fatal bleeding. Incised wounds of the neck can be accidental, homicidal, or suicidal. Accidental incised wounds are common and usually tend to be of a minor nature; hand injuries and distal fingertip amputations appear to be common among those using powered cutting equipment. Conversely, accidental fatalities due to working power tools are exceptionally rare. Suicidal incised wounds of the neck are typically multiple, often accompanied by a number of preliminary trial cuts (hesitation marks) and nearly all persons choosing this unusual manner of suicide suffer from psychosis or depression. In homicides the incision is single, extremely deep, and extends completely to the vertebral column. The scene of crime is suggestive of a struggle between victim and aggressor with disarray and blood everywhere.

The case presented concerns a 48-year-old man who was found dead in his own garage. The prosecutor's office was immediately alerted and a forensic pathology was involved in the crime scene investigation. The corpse was lying on the floor, and bloody stains were found everywhere around the cadaver. A circular saw with a smooth blade, 0.3cm thickness and 19.5cm diameter, was found on the cadaver and collected for examination. Evidence of a struggle in the garage was excluded as well as psychiatric disorders or unsuccessful suicide attempts in the past. A complete postmortem examination was performed a few days after death. At external examination, a wide, deep incised wound with regular margins was described crossing the anterior to the posterior side of the neck over and up the cervical bone's plane. Two superficial incised wounds were observed at the jaw and at the flexor surface of right forearm. Mild cerebral oedema was recorded at gross internal examination. Gross examination of the neck revealed massive hemorrhages in the subcutaneous tissues and muscles; left jugular vein, left common carotid artery, and left vagus nerve were isolated one by one and sectioned. A beautiful series of forensic autopsys pictures applied to the study of the face and the neck will be presented. The left thyroid lobe and left side of trachea showed lacerations. The paracervical muscles were hemorrhagic and a partial laceration of the fifth and sixth cervical vertebra was also detected. Examination of the heart and other organs was unremarkable except for a mild, white foam detected on the main bronchi. Small and big vessels were poor of blood. A complete histopathological study with Haematoxylin-Eosin (H&E) stain was performed. Hemorrhagic shock from transection of the left carotid artery and jugular vein was indicated as the cause of death.

The crime scene investigation and the distribution of lesions led us to conclude a rare case of accidental fatal injuries with circular saw. The peculiar technique used at autopsy to investigate injuries of the neck documented photographically will impact the forensic community and emphasize the importance of a careful crime scene investigation in order to differ between accidental, suicidal, or homicidal events.

Power Saw's Injuries, Crime Scene Investigation, Autopsy Technique

G78 Forensic Point of View in Manual Strangulation

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After attending this presentation, attendees will understand some principles of danger to life in manual strangulation.

This presentation will impact the forensic science community by the fact that the cause of death in manual strangulation is not yet elucidated.

Strangulation accounts for 2.5% of traumatic deaths worldwide and up to 10% of all violent death in the United States. Many people who are strangled survive. Much of forensic analysis and knowledge on manual strangulation was based on homicide victims. Serious damage to the vital structures in the neck was specific. Assessment of life-threatening injuries could be characterized as malicious intent.

All studies involving life-threatening and non-life threatening manual strangulation were reviewed. Are there any specific findings in clinical forensic medicine or in radiological investigation between deceased and living victims? Is the assessment of danger to life based on cerebral hypoxia and or cardiac reflex?

Only five studies were found in international literature. Only one, performed by a Swiss team, was clinical, all remaining studies were radiological. Three of them focused on the criteria between life-threatening and non-life-threatening manual strangulation. One compared strangulation signs between autopsy findings and multislice computed tomography and with magnetic resonance imaging. The clinic study proposed the categorization of strangulation victims who survived in three degrees of assault intensity (light, moderate, severe). It was stated that the duration of the neck compression would be associated with a high probability of a reflex cardiac arrest. Signs of typical manual strangulation prove the length and the forcefulness of the accused man. In contrast, radiological studies state the danger of life.

The appropriateness of life-threatening manual strangulation is crucial for forensic pathologists. The degree of penalty for the offender depends on this forensic point of view. Assessment of intensity and duration of manual strangulation by the injuries is still controversial when the cause of death remains obscure. There are four mechanisms of strangulation leading to death: venous obstruction leading to cerebral stagnation, arterial spasm due to carotid pressure leading to low cerebral blood flow, vagal collapse caused by pressure to carotid sinuses, and increased parasympathetic tone and obstruction of the airway by pressure on throat skeleton (unremitting manual pressure on the throat during four minutes seems practically unattainable). Mechanisms of death could be mixed in manual strangulation. The role of cardiac reflex by pressure on the neck in manual strangulation is still a controversial subject in forensic medicine.

Because of this, assessing life-threatening injuries is still difficult. When exactly does manual strangulation become a danger to life?

The clinical study explains that the classification should be considered as a proposition and forensic assessment is still individual. Radiological studies demonstrate the added value to forensic assessment by offering visualization of internal neck findings in victim survival or death. Forensic radiology studies concluded that the value of inner neck injuries is necessary. Forensic evaluation of danger to life needs reference standards and the small number of cases limits the results.

Further studies of survivors of manual strangulation have to be done. Comparative studies between forensic autopsy and forensic radiology must include more cases.

Manual Strangulation, Cause of Death, Danger to Life

G79 Cardioinhibitory Reflex Cardiac Arrest (CiRCA): Is it Possible Without Contributory Factors?

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After attending this presentation, attendees will learn that a single, short neck trauma can lead rapidly to death stimulating the carotid sinus cardioinhibitory reflex, without any compressive effect on the carotid arterial flow or any mechanical asphyxia.

This presentation will impact the forensic science community by highlighting that, as reported in the literature, there is no evidence proving that carotid stimulation alone can determine death.[1] In fact, in the present case, cardioinhibitory reflex cardiac arrest (CiRCA) resulted in the interaction between mechanical carotid sinus stimulation and underlying pathological and toxicological contributive factors.

It is widely known, in cardiology, that compression of the neck can produce variations in cardiac rhythm with bradycardia, syncope, and, in some cases, circulatory collapse resulting from the activation of an arterial baroreflex.[2,3] Remarkable interindividual variability in cardiac response has been reported.[4]

This physiopathological mechanism has been cited even in forensic setting, as the questioned cause responsible for deaths in which no other clear explanation is found. Many case studies have been recently reviewed[1] highlighting that reflex cardiac arrhythmia by neck stimulation is able to provoke death mainly with the contribution of other preexisting factors, such as drug abuse, cardiac diseases, and physical and/or mental excitation.

A 22-year-old Bangladese male, while spending time with some friends and other guests drinking alcohol in a night club, was dragged into a brawl outside of the establishment. Circumstances were carefully described by five different witnesses, all concordantly reporting that the man was hit by a single very strong punch on the left side of the neck, just below the mandibular angle. After the blow, the young man had a syncope and fell down, unconscious and unresponsive to intensive care.

Clinical records were examined in detail, including electrocardiograms. A history of chronic alcohol abuse, with multiple visits to the emergency for ethanol overdoses, was noted; apart from that, pathological anamnesis was normal.

Autopsy showed a remarkable bruise in the neck subcutaneous tissues and in the upper tract of the left sternocleidomastoid muscle, close to the neurovascular bundle. Further pathological exam ruled out any relevant traumatic brain or neck injury and cardiac disease.

Toxicology on postmortem blood specimens gave negative results for the most common drugs of abuse, except for the detection of ethanol 3.53g/L. Such blood alcohol concentration was similar to *in vivo* toxicological data of the subject, assessed during previous acute ethanol intoxications.

Forensic genetics were carried out screening mutations in three main genes (KCNQ1, KCNH2, SCN5A) involved in the Long QT Syndrome (LQTS). The deceased was a carrier of two different mutations in exon 11 of the KCNH2 gene, codifying for an ion channel named "HERG," related to the LQTs type 2. One was a silent mutation, but the subject was eterozygous for a missense mutation in

A2690C, responsible for aminoacidic substitution (from lysine to threonine) at codone 897 (K897T).

In this reported case, death by CiRCA was highly probable, but it was mediated by contributive factors, like alcohol intoxication and possible arrhythmic diathesis genetically determined. In this case, the high postmortem blood alcohol concentration does not explain death itself. In fact, in the literature,[5] lethal overdoses refer levels above 4.0g/L. Furthermore, tolerance to high alcohol levels by the subject, prone to repeated acute intoxications, must also be considered. More probably death resulted by mechanical activation of cardioinhibitory reflex at the neck with consequent bradycardia and loss of consciousness; then ethanol-related cardioinhibitory effects and brain depression led to final cardiac arrest. Questions remain as to the contribution of the genetic factor. In fact, while the detected missense mutation of KCNH2 gene seems to produce *in vitro* electrophysiological changes in sodium channel permeability,[6-8] its pathological significance *in vivo* is still uncertain.

In conclusion, the reported case confirmed that CiRCA should be a diagnosis by exclusion. It must be designated to cases in which circumstances are directly witnessed, and it should be supported by modern pathological, genetical, and toxicological analyses, to rule out other possible mechanisms of sudden unexplained death.

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CiRCA, Carotid Sinus, Neck Compression

G80 Spinal Cord Injury in Breech Labor

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After attending this presentation, attendees will understand how inadequate care of a breech delivery caused a perinatal death. The autopsy sheds light on how death occurred when extracting the fetal head and not by perinatal asphyxia.

This presentation will impact the forensic science community by higlighting how a multidisciplinary research involving the collaboration of academic peers and a group of forensic scientists was able to reconstruct the chain of events in an appropriate manner and how the factors that influenced this perinatal death were determined.

This is a case in which spinal cord injury occurred during a breech labor. According to a statement from the mother on the filed report, the mother stated that she felt her baby's body is coming out, but the physician failed to remove the head, so she thinks her baby's neck was broken. After spending a considerable amount of time, the full-term newborn was delivered without vital signs. The fetus's body was sent out for a forensic autopsy revealing the body of a female fetus of 3760g, 55cm height with cyanosis of the face. The internal examination anterior dissection revealed right parieto-temporal-occipital subgaleal hematoma, right parieto-occipital epidural hematoma, cerebral edema, and dislocation of a cervical vertebra. Posterior dissection performed up to the cervical region revealed dislocation of a cervical vertebra, spinal epidural hematoma from the first to the sixth cervical vertebra. After that, the spinal cord was lifted and samples were sent out for a histopathological study that revealed perimedullary spinal bleeding, brain and cerebellum micro stroke, and subarachnoid microhemorrhage. It is believed that this case of accidental death has never been reported.

This was unusual at that time because, initially, there were difficulties determining the cause of death due to the controversy that was stirred up among the physicians who attended the birth and the forensic autopsy pathologists. The medical autopsy revealed the findings from traumatic injury during breech delivery which caused the death of the full-term fetus in the vaginal canal, overflowing into the possible liability for lack of opportunity to perform a surgical procedure based on the absence of a pediatrician, arguments by the anesthesiologist, and the probable lack of expertise from the obstetrician/gynecologist specialists in the care of such deliveries. It is recommended that forensic investigators are familiar with this type of death as it is increasingly difficult to attend a breech delivery by technological advances in the institutions.

A case of perinatal death caused by spinal cord injury in the birth canal due to breech presentation associated with the delay in performing a cesarean section was reported. This case report describes, through a forensic autopsy with special dissections and review of medical records, the chain of events and pathogenetic mechanisms leading to the death of a fetus during labor. Medicolegal aspects of the case are discussed because when reviewing the existing literature, reports were found of the complications inherent to perinatal death due to breech presentation such as retention of the head, facial paralysis, and other injuries; but no reports on this type of death were found involving medical staff's negligence in the delay in performing a cesarean section.

Breech Delivery, Medical Liability, Spinal Epidural

G81 Overkill: A Report of Eight Cases and a Review of the Literature

Biagio Solarino, PhD, Giovanna Punzi, MD, Giancarlo Di Vella, MD, PhD, and Roberto Catanesi, MD, Univ of Bari, Piazza Giulio Cesare, 11, Bari, ITALY*

After attending this presentation, attendees will have a better knowledge of the overkill phenomenon and its significance from a forensic psychiatric rather than a forensic pathology standpoint.

This presentation will impact the forensic science community by emphasizing the need for a universally accepted definition of overkill, based on the importance of such an event from a criminalistic and juridical point of view.

It is not uncommon that homicides are perpetrated with multiple injuries inflicted when the victim has already died. This is indeed not sufficient for defining a case of overkill that deals with a peculiar manner of murder.

Only in a few articles or textbooks in forensic literature is the term overkill reported. In particular, some authors correlate overkill to sex-motivated homicides where injuries, generally stabbing, are directed to significant sexual parts of the body. These signs are consistent with

the psychodynamics of sexual sadism. Others refer to it as the infliction of massive injuries by a perpetrator far exceeding the extent necessary to kill the victim. This action involves a strong excitement of the offender who is possibly in deep personal conflicts with the victim.[1,2]

The psychopathic trait of a perpetrator is often evaluated in such cases, revealing some differences between "disorganized" and "organized" types of killers.

These peculiar features involve a careful scientific approach by the investigators in cases of suspected overkill. The medical examiner has the role of evaluating the body areas where the perpetrator inflicts injuries and the tool(s) used for wounding. Very importantly, the medical examiner may be able to ascertain if and when an offender understood the victim was already dead or received the ultimate lethal wound during an escalating fight. Hence, wound pattern analysis could be of a relevant significance in assessing the motive and intent of the offender in committing murder and overkill.

Forensic psychiatric consultation is of basilar importance for establishing the recurrence of the offender's psychopathy but also in helping resolve the manner of death. In cases of overkill, it is possible to face personality disorders as well as many forms of psychosis disease. Such an investigation allows one to understand the perpetrator's behavior at the moment of performing the criminal act, if they are mentally ill and require psychiatric hospitalization in custody, if they are dangerous for the general public, and, finally, for the assessment of juridical responsibility and related issues.

On these bases, eight cases of overkill were analyzed from both the medical examiner and forensic psychiatrist standpoint. Data about the victims (i.e., sex, age, occupation, cause, and manner of death) and the perpetrator (i.e., sex, age, occupation, relation to the victim) is provided and extensively discussed, as well as a criminological analysis of the motive and the historical setting of the homicides. The profiling of the perpetrators is enriched by the study of both the psychiatric history, before and upon the crime, and the legal history (psychiatric examination and sentence).

The data here collected will be discussed with regard to the elements from the crime scene and the postmortem, in an attempt to better understand the meaning of this peculiar *modus operandi*.

References:

1. Sex-related homicide and death investigation. Practical and clinical perspectives.Vernon J Geberth. CRC Press 2003.
2. Homicides by Sharp Force by Michael Bohnert *et al* in ForensicPathologyReviewsVolume 4, Edited byMichael Tsokos, Humana Press 2006

Overkill, Sex-Related Homicide, Forensic Medicine

G82 Extensive Hemorrhagic Retinopathy, Perimacular Retinal Fold, Retinoschisis, and Retinal Hemorrhage Progression Associated With a Fatal Spontaneous, Non-Traumatic, Intracranial Hemorrhage in an Infant

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After attending this presentation, attendees will learn that severe hemorrhagic retinopathy, perimacular retinal folds, and retinoschisis can occur from a fatal spontaneous, non-traumatic, intracranial hemorrhage in an infant. Attendees will also learn that retinal hemorrhages can progress in an infant during the course of hospitalization.

This presentation will impact the forensic science community by increasing the attendees' awareness of conditions in infants associated with severe hemorrhagic retinopathy, perimacular folds, retinoschisis, and retinal hemorrhage progression.

A number of studies continue to claim that Abusive Head Trauma (AHT) can be diagnosed with confidence when extensive Retinal Hemorrhages (RHs) accompanied by perimacular folds and retinoschisis are found in association with an intracranial hemorrhage in an infant. The only exceptions noted to date are crushed head injuries, or traumatic brain injuries associated with motor vehicular collisions. Natural disease processes causing intracranial hemorrhage have not been previously associated with severe hemorrhagic retinopathy, perimacular folds, and retinoschisis. Advanced as the explanation for the hemorrhagic retinopathy associated with AHT or Shaken Baby Syndrome, the vitreo-retinal traction theory assumes the RHs coincide with the intracranial injury and do not progress during hospitalization. A case of an infant with a fatal, spontaneous, non-traumatic intracranial hemorrhage associated with severe hemorrhagic retinopathy, retinoschisis, a perimacular fold, and RH progression was reported.

Case Report: A 2-month-old infant awoke from a nap fussy. Her mother changed her diaper and began preparing a bottle. Her father placed the infant over his shoulder and attempted to soothe her. A few minutes later he and the infant's aunt noticed that she was unresponsive. The paternal grandmother began cardiopulmonary resuscitation and on arrival of emergency medical services, the baby was apneic but had a palpable pulse. On presentation to the emergency department, she was hypotensive and started on a dopamine infusion. A cranial computed tomography scan demonstrated a large basilar subarachnoid hemorrhage occupying the supra-sellar cistern considered secondary to an aneurysm, arteriovenous malformation, or tumor. Blood was within the cerebral ventricles but because of the diffuse nature of the hemorrhage, the pediatric neuroradiologist could not rule out the possibility of non-accidental trauma. She was admitted to the pediatric intensive care unit and an osseous survey initially was interpreted as no evidence of acute or healing fractures, or other evidence of non-accidental trauma; however, on review of the skeletal survey remote healing/remodeling fractures involved the anterior left seventh and eighth ribs near the costochondral junction. A clinical examination consistent with brain death was confirmed by a negative cerebral blood flow study. Subsequently, an ophthalmology consultation with RetCam™ photo-documentation disclosed extensive multilayered RHs and a perimacular fold on the left, but no hemorrhages within the right fundus.

She had no external injuries and her autopsy was significant for severe cranial sutural diastasis, a large basilar subarachnoid hemorrhage, intraventricular hemorrhage, a right subdural hematoma, and severe anoxic ischemic brain injury, and cerebral edema. The spontaneous intracranial hemorrhage arose from an arteriovenous malformation of the choroid plexus adjacent to the hippocampus and inferior horn of the right lateral ventricle. Besides bilateral optic nerve sheath hemorrhages, she had diffuse multilayered RHs carpeting the left fundus to the ora serrata. A large premacular hemorrhagic cyst and perimacular fold with retinoschisis was present on the left, as well as vitreous hemorrhage. Although no RHs involved the right fundus during the first 21 hrs of hospitalization, she had subsequently developed multiple RHs over the right posterior pole while in the intensive care unit following a clinical brain death examination and negative cerebral blood flow study. Histologically, the healing fractures of the left ribs appeared at least four to six weeks of age.

This case describes severe hemorrhagic retinopathy with a perimacular fold and retinoschisis associated with a fatal spontaneous, non-traumatic, intracranial hemorrhage arising from an arteriovenous malformation of the choroid plexus. Also of significance, this infant had no RHs documented by a clinical ophthalmology examination and RetCam™ imaging while in the pediatric intensive care unit, but subsequently developed multiple multilayered RHs over the right posterior pole extending past the equator.

Although many authors consider that severe hemorrhagic retinopathy, retinoschisis, and perimacular folds are virtually pathognomonic of abusive head trauma, this case highlights the need for caution in attributing these ocular findings as diagnostically specific

for AHT. This case also emphasizes that RH progression can occur in infants during hospitalization.

Retinal Hemorrhage, Retinal Fold, Child Abuse

G83 Smartphone Image Acquisition During Postmortem Monocular Indirect Ophthalmoscopy

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After attending this presentation, attendees will learn how to use a smartphone to capture still and video images of projected aerial images produced during postmortem indirect ophthalmoscopy.

This presentation will impact the forensic science community by providing an overview of smartphone still and video image acquisition during postmortem monocular indirect ophthalmoscopy.

Postmortem Monocular Indirect Ophthalmoscopy (PMIO) permits examination of a decedent's posterior fundus facilitating detection of retinal abnormalities including Retinal Hemorrhages (RHs). The required equipment necessary for PMIO is relatively inexpensive; however, image acquisition of the projected aerial image typically requires a handheld fundal camera, which is prohibitively expensive for most medical examiner's offices. However, most smartphones have the capability of capturing the projected aerial image formed during PMIO. Smartphone image acquisition uses the smartphone's focal light source along with an aspheric convex indirect condensing lens. The light source of most smartphones can serve as the source of illumination if it is co-axial to the optical path of the camera's lens. Securing the smartphone on an adjustable mini-tripod permits the examiner to stabilize the condensing lens with both hands. Current aspheric lenses range from +14 to +40 diopters and come in different diameters permitting a field of view of 35 to 50 degrees. The condensing lens is pulled back slowly to image the fundus. High-quality still and video digital images of the projected aerial image are readily captured using this technique.

Various smartphone camera applications (apps) include tap screen focusing, zoom, voice or sound activated shutter release, and video recording, which facilitate high-resolution image acquisition. With the smartphone set on "camera" and the light source set to "on" for constant illumination, position the slider so the camera icon is activated. Position the condensing lens in front of the decedent's cornea making sure the lens, decedent's posterior retina, and camera are parallel. Slowly pull the lens toward the camera lens and light source to image the fundus. Since the projected aerial image is located near the focal point of the lens, it is necessary to tap-focus on areas of the thumb or index finger to bring the projected aerial image into clear focus. The camera's shutter is activated either by touching a screen icon or by a voice or sound shutter-release app.

To zoom in on an aspect of the image (up to 5x), pinch and drag to activate the zoom feature (put thumb and index finger together on the screen and drag them apart toward opposite ends of the screen). This will zoom in on the projected aerial image and reveal a slide bar at the edge of the image with a minus (-) on one end and a plus (+) on the other end. Zoom in or out for optimal composition by using the slide bar or pinch and drag.

Most smartphone cameras can also record video at up to 1080p HD. To change from taking still images to video, move the slider so that the button is under the movie camera icon and the camera will switch to video mode. To begin recording video, tap the button with the red circle in it. When recording, the red button will blink and a timer will appear onscreen. To stop recording, tap the button again.

Once recording of the projected aerial image is complete, the still images can be saved or the video images can be captured and edited from the video sequence. Various application programs enable users to upload still images or video sequences from the smartphone camera that is connected directly to a computer or network. Video editing software permits editing of images and video clips for documentation of the fundal findings. Still and video image acquisition using a smartphone during PMIO will be demonstrated. This technique has the potential to facilitate more widespread use of PMIO for fundus examination by forensic pathologists. Clinical application of the technique may permit telemedicine consultation, especially in poor resource settings.

Smartphone, Fundoscopy, Retinal Hemorrhages

G84 Airbag Injuries in Transportation Pathology

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After attending this presentation, attendees will achieve a better understanding of the injuries and the mechanisms of injuries related to airbag deployment.

This presentation will impact the forensic science community by highlighting how airbag injuries can be lethal and, sometimes, they are the only injuries responsible for death.

Airbags were invented more than 40 years ago. The goal of this presentation is to provide a better understanding of the injuries and the mechanisms of injuries related to airbag deployment. Airbags have proved to be a vital restraining device which is able to reduce morbidity and deaths associated with car crashes. The effectiveness of airbags in reducing fatalities and serious injuries is well established. However, due to the dynamic nature of airbag deployment, airbags are themselves a potential source of injuries and it has been estimated that more than 40% of airbag deployments result in a minimum of one airbag-related injury. Unlike other safety devices, airbags increase the amount of energy released during a car crash, thus they have always been regarded to be potentially dangerous to restrained and unrestrained occupants. Once considered very uncommon, airbag injuries are now reported more often, and the industry came to the conclusion that the airbag itself might be harmful and even potentially life threatening. The number of airbag-related injuries has proportionally increased with the number of vehicles equipped with airbags.

The expected pattern of injuries at autopsy can be complicated by airbag deployment. Airbags are intended to cushion the impact between the occupant and the interior structures of the vehicle (steering wheel or dashboard). Lateral airbags protect passengers from impact against side panels. The function of the airbag is to act as a "deformable object" in order to dissipate the greatest amount of kinetic energy possible. The majority of crashes in which airbag injuries occur are those of minor to moderate severity. During these events the seatbelt is regarded as providing a sufficient and adequate occupant protection. However, the unrestrained occupant might be exposed to forces which are greater than 18G.

The "bag slap" caused by the airbag deployment might be more harmful than the vehicular collision itself, especially in belted occupants. Recent studies have suggested a great reduction of fatality risk in belted drivers. However, other studies have linked the causation of some injuries—sometimes fatal—solely to airbag deployment.

Airbags rapidly reach their maximum pressure and dimensions by triggering a pyrotechnic device that inflates the bag in about 0.005 to 0.01 seconds. During deployment the airbag is propelled into the car compartment at a speed ranging between 157 to 338km/h. European airbags, which are designed to protect belted occupants, inflate to a volume of about 30 – 40 liters. They are smaller and deploy with much less force than American airbags, which are designed to protect both belted and unbelted passengers. American airbags deploy much more violently and inflate to a volume of 60 – 70 liters of gas.

All the constituents of the airbag module have been proved to be potentially harmful, comprising the airbag propellant capsule, the chemicals involved in the deployment, the inflating bag, and its

covering. The majority of injures are caused by the shearing forces following the rapid deployment of the bag, and are therefore related to blunt force trauma. As a consequence of the "punch out" of the inflating balloon, also known as "bag slap," the occupants may suffer fatal injuries such as skull fractures, fracture/dislocation of the cervical spine, lacerations of the liver, and severe chest trauma.

Airbag Injuries, Transportation Pathology, Road Traffic Fatalities

G85 Suicide by Double Gunshot Wounds From a Pen Gun: A Case Report

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After attending this presentation, attendees will be able to recognize a pen or a homemade gun as a firearm, to identify entrance wounds as a 0.22 long rifle bullet, and will understand how a low-energy bullet can be lethal.

This presentation will impact the forensic science community by exploring how an unusual firearm was used in a double-gunshot wound suicide.

In firearm museums, you can find ingenious systems like "pen guns" or "cane guns" that are known to be used for espionage. They are single-shot firearms.

Pen guns were primarily designed for the purpose of self defense or giving a signal. It is actually a homemade gun, adapted from an old-style cartridge pen or made by any competent machinist. But, harmless as it may seem, this is a device with deadly firepower. It is made for a personal use like self defense or criminal activity. It could be a surprising weapon with many accidents described in literature.

The design of the pen gun consisted of a housing looking like a writing pen, containing a spring-loaded firing mechanism. The ammunition used is a 0.22 long rifle manufactured cartridge.

Reported is a case of lethal injuries inflicted with a pen gun. This case represents an unusual firearm fatality reported to a weapon made to look innocuous.

A 49-year-old-man was found dead lying on his back on a mattress. To the right side of the body there was a pen on the floor. Inside the pen was a metallic cylinder, housing a spring-loaded metal rod; the chamber contained an empty 0.22 long rifle cartridge. Another empty 0.22 long rifle cartridge was found in the house, far from the body, on the stairs, with blood spatters on the walls. A letter was found close to the corpse. Standard X-rays showed two bullets in the left side of the chest. The external examination revealed the beginning state of decay. Upper extremities presented desiccation. There were two entrance wounds of 4mm in diameter surrounded by soot deposit. They were located on the chest 5cm to 6cm medial to the left nipple.

At autopsy, the first wound tract stopped in the abdominal musculature. It was a short tract, without bone, vascular, or visceral lesion. A non-deformed 0.22 long rifle bullet was found in the muscle. The second wound tract passed through the sixth left rib, through the pericardia and left ventricle, the trough inferior lobe of the left lung, and through the eighth intercostal space at the back. Autopsy found a hemopneumothorax, a hemopericardium, a left ventricle wound. A non-deformed 0.22 long rifle bullet was found in the musculature. The cause of death was massive bleeding. The manner of death was ruled as suicidal.

Primarily designed for self-defense, pen guns can be converted to weapons by simple means. Pen guns were not designed as assassination weapons. They would fail miserably in this role. They were conceived as clandestine escape weapons, not likely to be found during an initial search. It is surprising to observe fatal firearm injuries caused by pen guns. A 0.22 long rifle bullet is known to have low velocity and energy and their wounding capacity is consequently low. Generally, the projectile does not exit the body and the entrance wound is surrounded by a large amount of soot. The lethal potential of pen guns is real when fired at close range.

Pen Gun, Suicide, Chest

G86 Causes of Death in Hanging: A Review of Physiopathological Hypotheses From 1970 to 2010

Renaud Clement, MD, 1 Rue Gaston Veil, Nantes, FRANCE; and Lelie Meresse, MD, Chu de Nantes 1 Rue Gaston Veil, Nantes, FRANCE*

After attending this presentation, attendees will understand some principles of hanging physiopathological theories.

This presentation will impact the forensic science community by the fact that hanging knowledges are based on historic experimentations and few clinical observations

Filmed hangings have been used as a powerful tool in understanding the pathophysiology of human asphyxia. Before these new developments, most of our contemporary body of knowledge was in fact based on old writings from the end of the 19th-century and beginning of the 20th-century. In the present communication, the literature from 1970 to 2010 will be reviewed. It is important to understand the origin of our current theories and the models that were used to develop them and to inquire as to their validity. The exact mechanism of death has yet to be elucidated. For this study, medicolegal textbooks written in English and French were reviewed. The reading focused on the pathophysiological hypothesis accepted or rejected by contemporary authors: occlusion of the trachea, occlusion of vessels, and pneumogastric nerve stimulation. Experiments supporting the this studies positions were compiled, as well as their clinical observations. Thirteen medicolegal textbooks with a specific chapter of hanging were eligible. For each author, the principal mechanism of hanging death proceed to vascular occlusion. They base their positions on historic experimentations performed by Brouardel, Hofmann, and Minovici (for example). Polson and Di Maio reported their own observations to implicate vascular occlusion leading to death in hanging. Respiratory asphyxia could be mixed in as a possible mechanism of death, but it plays a minor role for all authors. Cardiac inhibition caused by the stimulation of pericarotid nerves during hanging is a theory that is accepted by seven authors as a possible cause of death. One author suspects this mechanism as a contributive role of death and five reject it. Nine authors provide a clinical description of hanging, and describe the loss of consciousness, convulsions, and apparent death. The onset of the loss of consciousness occurs earlier than 10 seconds. For all authors, the delay to convulsions is evocated without precision. The time of apparent death varied from three to five and ten to twenty minutes. The timing of three phases is not well estimated by authors. The description of convulsions and respiratory movements are lacking in evidence in all medicolegal textbooks. This historical review supports the fundamental scientific principle that just because a theory is accepted does not guarantee that it reflects the truth. In hanging deaths, respiratory asphyxia by occlusion of the airway was considered the principal cause of death for several decades before the theories of vascular occlusion and cardiac inhibition were gradually accepted. Current studies being carried out on filmed suicidal and autoerotic hangings demonstrate that respiratory obstruction is not the primary pathophysiologic phenomenon leading to death. Despite a long history of being investigated, the pathophysiology of hanging still needs to be revisited and studied in the 21st-century.

Hanging, Respiratory Asphyxia, Occlusion of Neck

G87 John Hunter's Treatise on Gunshot Wounds

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After attending this presentation, attendees will gain an appreciation of the state of knowledge of wound ballistics as published in the literature approximately one decade after the American Revolution.

This presentation will impact the forensic science community by explaining the earliest underpinnings of the foundational science of wound ballistics.

The first significant description of gunshot wounds in the medical literature was by the famed London surgeon, John Hunter, in his 1794 *Treatise on the Blood, Inflammation, and Gun-Shot Wounds*.

John Hunter is generally regarded as the "founder of scientific surgery" and is recognized as among the greatest medical pioneers in the history of medicine. He wrote four books and left many lectures and notes. His last book, *A Treatise on the Blood, Inflammation, and Gun-Shot Wounds*, is considered to be his most important. This masterwork was published in 1794, a year after his death at age 65. The *Treatise* is known as the first scientific study of wound healing, but it may also be the first publication on wound ballistics. The introduction and first three parts of the book discuss healing and inflammation and 63 pages of the fourth part are devoted to gunshot wounds and their treatment. He declared: "Little has been written on this subject [gunshot wound healing], although, perhaps, when we take every circumstance into consideration, it requires particular discussion; and what has been written is so superficial, that it deserves little attention." His discussions are the result of observations made in 1761 – 1763, from the age of 33, as Staff Surgeon during the Seven Years War (the French and Indian War); first, on the French Island of Belle Ile during the siege and capture of the French Belle Isle and second, in Lisbon during the defense of Portugal against the Spanish, as well as 30 years of study thereafter. He saw gunshot wounds as different from other wounds: "Gun-shot wounds are made by the projection of hard obtuse bodies, the greatest number of which are musket-balls; for cannon-balls, pieces of shells and stones from ramparts in sieges, or splinters of wood, etc. when on board of a ship in an engagement at sea, can hardly have their effects ranked among gun-shot wounds, they will come in more properly with wounds in general." Hunter advised against the prevailing desire to explore the wounds, but instead advocated against opening the wounds, recommending they be treated conservatively. Hunter believed that infection was a treatment failure and not an unavoidable stage of healing, as did his contemporaries. It was not until after Joseph Lister published his successful trials on antisepsis in 1867 that Hunter's radical views become accepted. Hunterian scholars have not previously commented on his discussions of wound ballistics. Hunter categorized gunshot wounds into simple and compound—the former when the missile passes through "soft parts only" and the latter with fractures of the bone, lacerations of the arteries, or penetration of the body cavities to include injury of vital organs. Hunter also described the effect of the velocity of the musket balls on wounds, to include the effect on the path of the bullet, the amount of damage, the rapidity of healing, and the fracture pattern of bones. Forensic pathologists will relate well when he writes in a section entitled *Of the Strange Course of Some Balls*: "The difficulty of finding balls, I have just observed, often arises from the irregular course they take. The regularity of the course of the passage of a ball will in general be in proportion to its velocity, and want of resistance; …"

The earliest description of gunshot wounds in the medical literature was published in the post-American Revolutionary period and was based upon clinical observations of musket balls during warfare.

Gunshot Wounds, Wound Ballistics, Forensic Pathology

G88 Determination of Genetic Profile After Visualization of Fingerprint Marks With Dactyloscopic Powders

Pamela Tozzo, MD, Alice Giuliodori, PhD, Daniele Rodriguez, and Luciana Caenazzo, Univ of Padua, Via Falloppio 50, Padova, ITALY*

After attending this presentation, attendees will learn the potential of DNA techniques to the analysis of latent fingerprints after visualization with dactyloscopic powder as an important application in forensic caseworks.

This presentation will impact the forensic science community by providing a new perspective of DNA analysis in forensic investigations.

With modern PCR-based technologies, it is possible to obtain a genetic profile from very small amounts of DNA. Due to this, intensive research has opened many new sources for forensic genetic investigation.

Since their introduction in forensic investigations, latent fingerprints recovery and analysis are still maintaining their importance, and the search and archiving of fingerprints is still one of the most important procedures during criminalist investigation. The powdering method is often used for visualization because it is easy, inexpensive, and gives immediate results. The technique relies on the mechanical adherence of fingerprint powder to the moisture and oily components of the skin ridge deposits. Unfortunately, sometimes it happens that the curve and loop patterns are unclear/incomplete, so the fingerprint may not be useful for identification purposes.

Today, latent fingerprints are not commonly used for DNA typing even if they could be considered useful DNA sources, as reported in previous publications which have demonstrated that even a single skin contact can transfer enough DNA for successful STR typing and that fingerprints are a possible DNA source for forensic DNA investigation.

The amount of DNA contained in a latent fingerprint was found to be independent of handling time, dependent on the individual handler and the substrate's characteristics. The success rate in obtaining a genetic profile (partial or complete) from a latent fingerprint will depend on the individual who has touched the surface (good or bad shedder), the activities of the individual prior to touching the substrate, and the nature of the substrates. In forensic casework, neither the shedding nature of the individual nor the activities of the individual prior to touching the object are usually known.

To help address these aspects, a study was conducted on the effect of fingerprint enhancement methods on subsequent STR profiling.

First, a systematic study typing blood traces deposited on five different surfaces, both porous and non-porous, treated with eight types of dactyloscopic powders was performed. Three different DNA extraction methods were used.

In the second part of the study, the possibility of obtaining DNA profiles from latent fingerprints on the same five surfaces enhanced with the eight different powders used in the first part of the study was analyzed. On fragments of the cotton swabs used for recovering DNA from latent fingerprints, extraction procedures with two methods were performed. In order to obtain the greatest number of interpretable profiles, they adopted strategies in the extraction step, using techniques that would guarantee the more purified eluted DNA as possible, and in the amplification reaction, improving its sensitivity compared to the small amount of isolated DNA. Considering the low amount of DNA contained in latent fingerprints, all PCR reactions were carried out three times to obtain a consensus genetic profile.

The work in this study has demonstrated that DNA profiling can be performed on fingerprints left on different substrates and the nature of the substrate will affect the amount of DNA that can be recovered for DNA typing analysis.

In the first phase of the study, a profile was obtained in 92% of the 120 samples analyzed, with the percentage of full profiles of 60%; in the second part, in 55% of the 80 samples analyzed the authors

obtained a profile, complete in 32.5% of cases. The substrates that have led to the largest number of profiles have been the metal and the glass, probably due to the lower adhesion of the powders to these surfaces.

From the results obtained, it seems that the powders used in latent fingerprints enhancement, rather than having a direct inhibitory effect on extraction and amplification of DNA, may cause partial degradation of DNA, thereby reducing the efficiency of amplification reaction.

Genetic Profile, Latent Fingerprints, Dactyloscopic Powder

G89 Medications Error: Easy and Frequent Occurrences of Events but Difficult Reconstruction and Demonstration

Federica Colosimo, MD, Luca Gallelli, and Giulio Di Mizio, PhD, MD, Magna Graecia Univ, Viale Europa Loc. Germaneto, Catanzaro, ITALY*

The goal of this presentation is to show how medication errors represent preventable incidents occurring at any point in a chain of events beginning with prescription writing and continuing on to both drug dispensation and administration.

This presentation will impact the forensic science community by serving as a key aspect during drug dispensing; in fact, it is possible that a better counseling could reduce the risk to develop adverse events.

Several data have been published regarding this problem. In fact, it has been estimated that medication errors account for about 78% of serious medical errors in intensive care units; it is important to underline that a medication error can involve drug-related problems during the homecare setting. In particular, a recent paper has estimated that up to 30% of homecare patients experience a potential medication error, happening during preparation and administration of drugs. Medication errors can also induce a decrease in patient health status with an increase in health-care costs and occurs at every stage of the drug delivery process, i.e., prescribing, transcribing, dispensing, and administering.

However, the risk of the development of a serious disease induced by a medication error could be related to three factors: polytherapy, age of patient, and the therapeutic index. In fact, polytherapy could induce the development of drug-drug interaction; elderly patients present a physiological decrease of drug metabolism and drug excretion, responsible for drug accumulation; finally, the therapeutic index represents the safety of each drug; therefore, a medication error related to the administration of a drug with a very low therapeutic index (e.g., digoxin, theophylline, or anticancer drugs) could easily induce a decrease in health status.

Cases of personal observation will be presented in which medication errors have caused damage to the patients, or even death. They also perform the analysis of organizational remedies feasible with good clinical practice of risk management. Finally, the possible consequences of trial relating to these events, both in criminal court and in the compensation of damages, will be addressed. Therefore, it becomes an important aspect during the discussion on the methodology of forensic evaluation of the damage, for which it is crucial to establish a causal link between incorrect drug dosage and development of damage. The forensic criteriology based on Italian legal doctrine provides an adequate response to the need to perform, in such cases, assessments based on both strict survey methodology and laboratory protocols scientifically validated. The above methods could reduce the maximum margin of error and allow rule out other causes of disease. The medicolegal criteria that should be demonstrated to establish a causal connection between medication error and damage involve four major criteria: *anamnestic* criterion (e.g., the history of the patient), *clinical* criterion (e.g., clinical signs or symptoms and biochemical markers of disease), *anatomo pathological* criterion (usually performed on a cadaver, aimed at macroscopic and microscopic morphological recognition of specific target of organs and tissues due to drugs), and, finally, *toxicological* criterion (that is an "objective criterion" involving the identification and isolation of the substance given).

Medication Error, Clinical Risk Management, Clinical Pharmacology

G90 CT Multislice Guided Autopsy Methodology in a Fatal Body Packing Case

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The goal of this presentation is to provide information to show how acknowledged cases of body packing are quite rare and the phenomenon often goes unrecognized. Fatal cases are even more infrequent, despite the fact that drug abuse is a growing social phenomenon definitely worthy of notice. That's why further study of this subject is necessary in order to be able to recognize and adequately treat the specific case should the need arise. When presented with unexpected deaths of young persons coming from specific areas, we should actually take into account the possibility of drugs being transported, in order to perform preliminary instrumental examinations that may be helpful for a proper autopsy execution.

This presentation will impact the forensic science community by showing how instrumental examination, such as X-ray, CT scan, and RMN can be very useful as a complementary survey to the autopsy. These techniques of investigation should also be more important in specific and difficult cases, such as in advanced decomposition of bodies.

Objectives: Within the field of forensic medicine, acknowledged cases of body packing are quite rare and the phenomenon often goes unrecognized. Fatal cases are even more infrequent, despite the fact that drug abuse is a growing social phenomenon definitely worthy of notice. That's why further study of this subject is necessary in order to be able to recognize and adequately treat the specific case should the need arise. When presented with unexpected deaths of young persons coming from specific areas, we should actually take into account the possibility of drugs being transported, in order to perform preliminary instrumental examinations that may be helpful for a proper autopsy execution.

Case: In the autumn of 2012, a young Black man coming from Tanzania was found dead in his hotel room. The man had been rescued at a train station for symptoms compatible with opiates poisoning and treated with Naloxone. His health rapidly improved, so he refused further medical exams and left the hospital, against the will of doctors. Before the autopsy, a multi-slice total-body CT scan exam was performed, which showed the presence of a lot of ovoid-shaped objects distributed across the man's whole gastrointestinal tract, from his stomach down to his rectum. The gastrointestinal tract dissection revealed 87 ovoid packets, which were extracted and handed over to the police for further forensic analysis.

Materials and Methods: A CT Somaton 16 Slices was used to perform a Computed Tomography (CT) scan with a 3D reconstruction. Gas Chromatography/Mass Spectrometry (GC/MS) was used to perform biochemical analysis on the packets' contents. GC/MS, High Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD), and Headspace Gas Chromatography Flame Ionization Detector (HS-GC-FID) were used to quantify free heroin and other drugs in blood and fluids.

Then histological preparations with seven micron sections were performed and, after that, stained with Haematoxylin and Eosin.

Results: After the autopsy findings, in the local Scientific Police Station, detailed biochemical analysis were performed in order to find out the content of the 87 ovoid packets. Each of these packets contained approximately 15g of light brown powder, which proved the subsequent analysis contained heroin, at the medium concentration of 14.8%. It was found, therefore, that the degree of purity of this substance was much lower compared with literature data. In fact, in drug smuggling, publications made by authors of different countries all over the world show degrees of purity of heroin in the range of 50 – 90%. A drug screen conducted on specimens of blood, received from the autopsy, found lethal values of opiates (in the urine there was a total morphine concentration of 45000µg/l and a codeine concentration of 3700µg/l; in blood samples there was a total morphine concentration of 220µg/l and a codeine concentration of 22µg/l). The toxicological analyses also revealed the presence of acetaminophen in the urine and caffeine in the blood. In conclusion, these evidences show that the man died as a result of heroin intoxication.

Conclusions: In this case report, the preliminary execution of CT scan, performed before the autopsy, was very helpfull in substantiating that the man was a drug smuggler and showed exactly where to find the ovoid packets in the body, so as to guide the forensic pathologist during the autopsy, also facilitating the preservation and collection of the packets. This technique has been underutilized and poorly reported in literature, especially the 3D reconstruction, which has been so helpfull in this particular case report. Instrumental examination, such as X-ray, CT scan and RMN, can be very useful as complementary survey to the autopsy. These techniques of investigation should be more important in specific and difficult cases, such as in advanced decomposition of bodies.

Body Packer, Heroin, CT Multislice

G91 How to Scan Hot Stuffs: Scanning Electron Microscopy Applied to Forensic Investigations

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After attending this presentation, attendees will discover that scanning electron microscopy has a wide number of possible applications in forensic investigations. Attendees will also have a general understanding of the advantages of scanning electron microscopy in forensic analysis. They will be shown that the Scanning Electron Microscope (SEM) is not only a mere tool, but is an actual method of forensic investigation which requires operators to see the possibility of performing this analysis at the time of autopsy and/or when samples are collected.

This presentation will impact the forensic science community by demonstrating various methods in which organic and inorganic samples may be analyzed with an SEM and by providing elements to better understand the evolution and application of this technology to forensic cases.

Since it is a vesatile instrument, the SEM is widely used in forensic investigations. It is used to study a large variety of specimens in forensic investigations such as the analysis of gunshot residue, bullet fingerprints, bullet wipe or patterns around the bullet hole, environmental dusts, fibers (both natural and artificial), and ink and paper analysis.

In this presentation, a selected number of investigations are shown, to illustrate through specific cases, general purpose applications: SEM has the ability of providing both panoramic and highly magnified views of the same sample, giving an almost 3D view of the specimen. It is the ideal *trait d union* between macroscopic information collected during autoptic or investigative activity and microscopic information obtained with the light microscope. Above all, the SEM is an instrument that allows to perform a progressive and targeted microdissection of the sample, possibly at the time of autopsy and/or the collection of the sample.

This presentation will focus on the experience gained over the past four years by the Forensic Laboratory, a multi-discipline unit of the University of Insubria. At the Human Anatomy Laboratory, the sample, collected during the forensic investigation, is reduced, when necessary, to an appropriate size to fit in the specimen chamber, then prepared to be observed under the scanning electron microscope (FEI Philips XL-30 FEG-SEM microscope).

After a preliminary analysis, usually made by both secondary electrons detection (which shows the morphology of the sample surface) and detection of backscattered electrons (since high atomic number elements backscatter electrons more strongly than low atomic number elements, back scattered electron images show areas with different chemical composition as zones of different brightness over the sample), the sample is prepared once again: it is subjected to a osmium tetroxide maceration post fixation process in order to enhance membrane contrast and to better visualize intracellular structures and/or it is prepared by NaOH 1N maceration, when a better evidence of connective tissue stroma is desired. In these cases, the specimens are mounted on adhesive films applied on standard aluminium stubs, gold coated in an Emitech K550 sputter coater (Emitech Products Inc.) and then observed under the scanning electron microscope.

When the situation requires it, energy dispersive X-ray spectroscopy and X-ray mapping of specimens are performed, to identify the elemental composition of the whole sample or small area of interest on the sample.

SEM, EDS Microanalysis, Bloodstains

G92 A Singular Case of Asphyxia by Choking With a Handkerchief: Accidental Event or Suicide to "Shut-Up" Spirits?

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After attending this presentation, attendees will understand the importance of integrating circumstantial data, clinical history, and autoptic results in the differential diagnosis between homicidal, suicidal, or accidental death by choking.

This presentation will impact the forensic science community by providing key information that can help to correctly evaluate rare asphyxia cases.

Suicide accounts for 3% of deaths in the world's population. The SUPRE Project (Suicide Prevention Project - OMS) estimates that from 1950 to 1995 the percentage of deaths by suicide has increased globally by 60% and is currently growing, especially among the younger age groups.

In Italy, every year there are between 3,500 and 4,000 suicides; most of these are made by patients with psychiatric disorders. These numbers rise if a psycho-pathological state is associated with the abuse of substances such as alcohol and drugs. Among the psychiatric disorders, subjects with schizophrenia are particularly at risk.

Individuals with schizophrenia have many basic functions compromised, which include perceiving, thinking, language, emotions, will, initiative, and attention. The impairment is likely to lead to serious problems of adaptation in social or occupational functions, and it influences the decision-making capacity, pushing patients to take extreme acts.

Severe psychotic symptoms, such as delusions or hallucinations, and pathological personality traits, such as impulsivity and excessive suspiciousness, are additional factors that can determine an increase in the number of suicide attempts and in fatality.

Suicide by choking is extremely rare. The case presented appeared remarkable for the way in which it was set in place by the victim, the reasons why the victim attempted suicide, and doubts about the nature of the act.

In January 2010, a 60-year-old woman was found dead in her home with a handkerchief down her throat. The victim a had longstanding history of psychiatric illness (residual chronic schizophrenic psychosis in partially effective neuroleptic treatment). The victim had been under investigation by prosecutors for fraud for selling her services as a "sorceress." The son also reported previous suicide attempts made by his mother, even in his presence, guided by evil spirits' voices coming from inside her body. In order to "shut-up" the spirits, she tried to suffocate them with her hands or by self garroting with a belt. The external examination of the body showed abundant red wine hypostasis localized on face, neck, and upper chest. Autopsy showed petechiae in visceral pleura and epicardium, and visceral congestion. The absence of the upper left canine and lower left premolar with no traumatic lesions of the labial mucosa was also noted. There was no other sign of internal or external trauma. Internal organ sections showed hemorrhagic infiltration at the soft palate and at the soft tissues of the upper portion of the larynx. The histological examination confirmed multi-organ congestion and subpleural emphysema. Toxicological findings denoted and quantified clozapine overdose with a consumption over three times the prescribed dose (300mg/day).

Suicide by choking is difficult to accomplish and can easily be confused with accidental or homicidal choking. The psychotic factor is an important variable to consider in the suicidal behavior, especially for the hallucinatory component, which most likely induces some patients to perform acts motivated by a distorted view of reality

In the case presented, there were two possible scenarios, the first in which the patient, suffering from a psychotic delirium characterized by voices coming from inside, decided to end it by suffocating and purposefully placing tissues down the pharynx. In the second scenario, the patient placed a handkerchief in the mouth on the upper left canine to buffer the bleeding, but being under the influence of the clozapine (side effects: drowsiness, mental confusion, agitation, respiratory depression), airways were fatally blocked, causing a violent mechanical asphyxia.

Choking, Clozapine, Handkerchief

G93 Suicides Due to Substance Overdose in Tarrant County, Texas

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After attending this presentation, attendees will learn the incidence of suicides due to substance overdose in Tarrant County, Texas, from January of 2006 to December of 2010, and its comparison to larger studies.

This presentation will impact the forensic science community by increasing awareness that prescription drugs are the leading substance type used among individuals who died by suicide due to substance overdose and that cautious prescribing practices are essential.

Introduction: Suicide is the 11th leading cause of death of all ages in the United States and more than 34,000 people end their lives every year. Suicide is found in every age, racial, and ethnic group. Center for Disease Control and Prevention (CDC) reports that substance overdose is the third-leading method of suicide, following firearm and hanging/strangulation. The vast majority of substance overdose suicides are related to prescription drugs. The purpose of this study was to summarize and demonstrate the characteristics of suicide deaths due to substance overdose in Tarrant County, Texas.

Material and Methods: This study included all suicide cases due to substance overdose in Tarrant County, Texas, from January of 2006 to December of 2010. Data were collected from the Tarrant County Medical Examiner Office records retrospectively. The substance was grouped into prescription drugs (benzodiazepines, opioid analgesics, antidepressants, and others), over-the-counter drugs, illicit drugs, ethanol, and other substances. The data were analyzed according to the age group, gender, race, and numbers and types of drugs.

Results: There were 90 eligible suicide cases (48 males, 42 females) due to substance overdose out of 733 substance-related deaths. The eligible cases represent 10% of all suicide cases (90 out of 891) during the same period. The majority of cases were Caucasians. The highest suicide rate (37%) was observed between ages 45 and 54, with the most cases (78%) falling between ages 25 and 54. Prescription drugs (75%) were the leading substance type used, with antidepressants the most popular. Thirty-eight percent ingested only one type of substance and 62% ingested two or more types of substances. There was no considerable difference between males and females in a total number of the suicide cases; however, the majority (79%) of females, compared to about half (48%) of males, ingested more than one type of substance.

Conclusions and Discussions: Suicide is a serious public health concern and substance overdose accounts for numbers of suicides. This study demonstrates an increasing problem with suicide deaths due to fatal overdose of prescription drugs in Tarrant County, Texas, a finding similar to other larger studies. However, more individuals (62%) ingested two or more types of substances in Tarrant County compared to a recent CDC report (25%). Many of these deaths can be prevented by controlling access to poisonous substances, and many of the substances used in suicides are commonly prescribed. Physicians and other clinicians should strictly follow guidelines for prescribing drugs with a high abuse potential. Surveillance systems designed to prevent illicit use of prescription drugs need to be implemented. Effective drug therapy is necessary for physical and mental health conditions; however, health care providers, patients, and their family members should be aware of the associated risk these substances pose.

Drugs, Overdose, Suicide

G94 Age Estimation Using T-Cell Receptor Excision Circles (TRECs) in Forensics

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After attending this presentation, attendees will understand the basic principle of formation of T-Cell Receptor Excision Circles (TRECs) and its change during a lifetime. The feasibility of its use in forensic practice and ethnic difference, if it ever exists, will be discussed and compared with previous reports.

This presentation will impact the forensic science community by providing evidence or techniques using the relationship between TRECs levels and age.

Age estimation using biological remains is one of the hottest topics in forensics. Many different approaches have been tried up to now, and the age estimation using TRECs is gaining interest.

The central role of thymus in the production of T-cells and the generation of T-cell receptors (TCRs) including TCR gene rearrangement is well established, together with thymic changes with time such as thymic involution. During the rearrangement of TCR gene segments, some regions which were not selected to form the parts of TCRs are spliced out as ring-shaped DNA. This exists in naïve T-cells immediately after development and maturation in thymus. Signal-Joint T-cell Receptor Excision Circle (sjTREC), which is one of the by-products of the rearrangement of gene segments encoding the variable parts of T-cell receptor α and β chains, not only

replicates during cellular proliferation in the periphery, but also is diluted with each round of cell division. Therefore, it is supposed that the content of these episomal DNA per total number of T-cells or level of constant genes would decrease with aging. In forensic cases, this biological phenomenon could be useful for providing evidence using the relationship between quantitative sjTRECs level and age.

For a long time, measurement of thymic output was indirect, mainly based on the phenotypic markers on naïve T-cells. However, since it is essential to detect the level of TRECs in the peripheral blood precisely and sensitively, it is necessary to utilize the sequence-specific DNA detection method. One of the most promising candidates is real-time quantitative PCR assay. Primers were designed to detect TRECs by targeting only the excised sequences after TCR gene rearrangement. Furthermore, TREC fragment-cloned plasmid was obtained, which is necessary for positive control and quantification using a standard curve. Using this plasmid sensitivity of the test was confirmed. Meanwhile, the immunological conditions such as blood volume or number of T-cells affected by immunological diseases or virus infections vary per person. These conditions also influence the relative level of TRECs in periphery blood. To normalize their level, it was observed that some genes can reflect these conditions or are expressed constantly, such as human serum albumin known to exist in blood plasma, or TCR alpha chain known to be a constant region of T-cell receptor. If it is necessary, various analysis methods which were utilized in other reports will be introduced.

This method is based on an immunologic principle, so the results would be variable depending on different ethnicities. After obtaining ethics committee approval, DNA samples from random Koreans of varying age groups would be tested for the above method. These results would be compared with others previously reported for different populations.

Age Estimation, TRECs, Forensics

G95 When Sadistic Fantasies Are Turned Into Reality

Francesco Vinci, MD, Felice F. Carabellese, MD, Maricla Marrone, MD, Chiara Candelli, MD, PhD, Francesco Rodriguez, and Roberto Catanesi, MD, Univ of Bari, Piazza Giulio Cesare, 11, Bari, ITALY*

After attending this presentation, attendees will understand the extreme variability of the psychological drives of perpetrators of heinous sexual crimes.

This presentation will impact the forensic science community by connecting the relationship between sexuality and aggressiveness.

A case of murder of an 89-year-old woman, found dead in her home, is reported. The woman's body was prone and partially naked (from the lower half of the trunk down), with legs apart, pelvis and groin resting on the edge of the bed and a nightdress wound around her neck as a noose.

Examination of the body allowed many bruised areas of various shapes and sizes located in different parts, as well as protrusion of the tongue to be identified. Examination of the vulva highlighted the presence of a small, oval tear in the vicinity of the frenulum of the labia majora and along the median line. The hymen was infiltrated with blood and on the skin around the anal sphincter there were other bleeding tears.

In addition to infiltrated deep bleeding at some of the ecchymotic areas above described, the autopsy revealed the presence of blood infiltrates of the internal structures of the neck (sternocleidomastoid, root of the tongue, pharynx, and larynx, right jugular vein, and left carotid artery), fracture of the greater horn of the right hyoid bone with hemorrhagic infiltration of the carotid artery intima, that was fissured. These signs are all compatible with compression of the neck and death by asphyxia. Confirming this hypothesis, there was blood within the vascular tree, congestion of the viscera, emphysema, and pulmonary edema, as well as rare, small ecchymoses of serous membranes. The cause of death was therefore defined as asphyxia due to violent mechanical acute strangulation.

On the body of the victim, there were also numerous injuries, some of which were correlated to generic trauma and others to sexual assault. As regards the former, they suggested a prior altercation with her assailant, whereas the injuries orienting toward a sexual assault were clear signs of both vaginal and anal penetration, as well as bruises and abrasions present on the lower limbs. This hypothesis was supported by the finding of sperm inside the rectum. The evidence concluded that the elderly woman was the victim of a violent anal assault while she was forcibly held prone, probably in the same position as she was later found dead.

Considering that the anal lesions were made while the woman was alive, it could be assumed that the strangulation had occurred after the coitus; moreover, the particular position of the knot and of the ends of the noose (at the victim's nape) suggested that the assailant was behind the victim. As no sperm was found in the vagina, the presence of a bleeding hymen and a small laceration of the labia majora led us to the supposition that there had been vaginal penetration only in the early stages of the rape, which was then ended in the rectum.

Thanks to the finding of shoe prints on the wall of the victim's home, the subsequent investigations quickly led to the arrest of a 22-year-old who admitted responsibility but was unable to provide any plausible motivation for the criminal conduct and violent assault.

Through a dynamic reconstruction of the crime and clinical analysis of the aggressor's personality, the role of sexuality when conceiving the crime was reconsidered. Despite appearances, it became clear that the sexual violence was the expression of aggressiveness. This aggressive behavior aimed against the elderly woman was performed in the form of destructive violence and sexual desecration. These considerations became the foundation of a close psychiatric and forensic evaluation from which it emerged that, in this case, there were no detectable elements linked to a mental disease, even temporary, but only expressions of a disturbed personality in which an altered affectivity played a significant role.

Sadistic Fantasies, Homicide, Forensic Evaluation

G96 A Review on Italian Mafia Homicides: "Men of Honor" and Ritual Crimes

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The goal of this presentation is to present typical homicide modalities practiced by organized crime syndicates in Italy. The killing styles operated by prominent criminal organizations vary from one group to the next, and it could be argued that different cultures, dependent upon geographical location, affect criminal behavior. Some forms of homicide are quite particular and their significance is still very often misunderstood.

This presentation will impact the forensic science community by influencing the judicial history of Italy through illustrating scenarios relating to Italian Mafia murders and a unique collection of images.

Homicides perpetrated by organized criminal groups are typically very brutal in nature. They often involve "special rituals" charged with symbolic meaning, which may be more or less explicit, depending on the chosen manner of execution and on the victim. Throughout the world, the term "mafia" is associated with organized criminal groups of Italian origin; these hierarchically arranged family groups are controlled by high-ranking members who make the decisions which are then passed down the hierarchy to other members of the family. The Mafia is not a single group or gang but it is made up of many families that have, at times, fought each other in bitter, bloody gang wars. At other times, they have cooperated in the interest of greater profits, sometimes even serving on a "Commission" responsible for

making decisions that affect all the families. It could be argued that Italian organized crime is not comparable to any other existing forms of criminal activity. Criminal associations such as the *Camorra,* originating from the region of Campania, *Cosa Nostra* based in Sicily, the *Ndrangheta* of Calabria, and the *Sacra Corona Unita* whose native land is the region of Puglia, are largely responsible for the development of different kinds of organized crime, not only in Italy, but with tentacles reaching outside the country. The culture and traditions of the different regions of origin affect the chosen modes of execution. This study moves through a preliminary narrative of the history and origins of each criminal organization, their main illegal activities, and finally onto some of the typical homicidal modalities, amply documented with a rich and varied collection of photographic material gathered during the investigation of crime scenes and from autopsies.

A specific case of a Calabrian mafia-related homicide is execution perpetrated by a single shotgun shot to the head followed by the deliberate concealment of the victims body, or the use of caustic substances to erase or modify features (facial), thus rendering the cadaver unrecognizable. In the cases presented, cadavers wore gloves to preserve fingerprints to aid in identification. The Apulian Mafia (the *Sacra Corona Unita*) has a similar operating technique; after shooting their victims, the corpse is buried in an abandoned area. This study looks at the case of four decomposed corpses discovered in cars plunged into ravines. In other cases, shooting is followed by charring of the body, making it impossible to recognize the victim without the use of genetic investigation. Radiological investigations (X-rays, MSCT) performed before autopsies are indispensable for the detection of bullets. Multiple cases of *incaprettamen*to (homicidal ligature strangulation) are also presented in this study. This particular ritual can be considered the hallmark of the Sicilian Mafia (*Cosa Nostra*). The victims wrists and ankles are pulled together behind his back, and an additional rope is tied around the neck and attached to the bindings in such a way that any attempt to free himself from this unnatural, contorted position will eventually provoke death by self-strangulation. One of the reasons for this homicidal mode is for the ease of transporting the corpse, which is often found inside the trunk of the car used for transportation.

Murder techniques used by the local mafia in the Gargano, a geographical area in the north of Apulia were also described. Here the manner of execution is characterized by the explosion of multiple shots from firearms (shotguns), some of which exploded in the face, serving the double purpose of killing the victim and simultaneously scarring the body, which would later be seen by relatives.

Italian Mafia, Homicide Modalities, Lupara Bianca

G97 Hantavirus Pulmonary Syndrome: A Case Report

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After this presentation, attendees will be able to describe the geographical distribution of Hantavirus, its host, routes transmission,detail the pathogenesis and symptomatology infection, and characterize the Hantavirus Pulmonary Syndrome (HPS), and describe expected autopsy findings

This presentation will impact the forensic science community by familiarizing its audience with the features of the HPS, its clinical presentation, autopsy findings, and pertinent postmortem ancillary testing

Hantaviruses include a related group of RNA viruses of the Bunyaviridae family. It is transmitted by rodent hosts, mainly via aerosolized feces and urine, causing only incidental disease in humans. HPS, however rare, is a serious life-threateninig condition that needs early diagnosis and support therapy, since symptoms can develop very rapidly (within 24 hours).

Presented is the case of a 20-year-old woman with no significant past medical history who complained of having headache, cough, muscle and stomach aches, fever, and shortness of breath for several days. She was seen at a clinic and treated as an outpatient with ciprofloxacin. Two days later, she was taken to an urgent care facility due to acute onset of shortness of breath. She was admitted to the Intensive Care Unit (ICU) and died shortly after her admission. The admission Computed Tomography (CT) scan showed extensive bilateral pleural effusions.

Autopsy revealed marked lung edema and large bilateral straw-colored pleural effusions (right, 850mL; left, 550mL). Microscopically, the lungs showed extensive areas of intra-alveolar fluid, with fibrin depositiona and focal hyaline membrane formation. Anterstitial mononuclear cell infiltrate with immunoblasts was identified in the lungs, liver, and spleen. A serum assay was positive for *Sin Nobre* Hantavirus antibodies.

While the incidence of Hantavirus infections has been relatively low (551 cases reported in the United States between 1993 and 2011), the mortality rate is alarmingly high (about 35%). This is due to a virus-induced vigorous immune response within vascular epithelium leading to vascular instability, especially within the lungs. Capillary dilation and leakage lead to the more severe manifestations of disease: shock and respiratory failure.

Without an intermediate host, each rodent host serves as both the primary host and reservoir in nature, transmitted via saliva, respiratory tract secretions, feces, and urine, causing chronic infection in rodents and only incidental disease in humans, generally by inhalation of aerosols of these body fluids or of virus-laden dust. Other means of infection include bites from infected animals, contamination of cutaneous wounds or mucous membranes, or ingestion of contaminated food. With the exception of the Andes virus of Argentina, the virus is not transmitted between humans.

At particular risk are individuals living or working in small, dark spaces with poor ventilation and known rodent presence, perhaps following a period of environmental conditions favorable to rodent reproduction. A classic case might describe exposure following cleaning of a shed, vacation home, or livestock feed container.

The natural history of disease of HPS generally includes three phases that follow an incubation period that lasts from two to four weeks: prodrome—characterized by sudden onset of nonspecific fever, malaise, myalgia, headache, chills, nausea, or diarrhea, and lasting about three to five days; cardiopulmonary—with nonproductive cough and varying degrees of shortness of breath, with associated tachypnea and mild hypotension and, laboratory evaluation—will reveal hemoconcentration (elevated hemoglobin/hematocrit), thrombocytopenia, neutrophilic leukocytosis with left shift, and reactive lymphocytes. At this stage, the patient is at the most risk for massive fluid shifts and pulmonary edema. The patients who die usually succumb to progressive cardiac insufficiency. If this phase is contained, the convalescence—or diuretic phase—begins, with usually little significant long-term sequela, though exertional dyspnea may persist for up to three months.

Considering the speed with which Hantavirus can overwhelm its victims, rapid diagnosis is essential in the clinical setting. In the postmortem setting, this is no less true, as a quickly recognized and diagnosed Hantavirus infection can allow medical investigators to identify family or coworkers at risk of a similar decline.

Serology remains the most common means of Hantavirus diagnosis, with IgM-capture ELISA providing a presumptive diagnosis. This can be performed in the postmortem setting, but the authors recommend making every effort to get an antemortem sample, since serum extraction in postmortem samples can become difficult. Detection of hantaviral antigen in tissue biopsy or other autopsy specimens can also be pursued. Other diagnostic modalities include viral RT-PCR from blood or fixed tissue or immunohistochemical demonstration of viral antigen in fixed tissue.

Hantavirus, *Sin Nombre* Virus, Pulmonary Syndrome

G98 Workplace Fatalities: A Case Example of Asphyxia Due to Occupational Exposure to Airborne Chemicals

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After attending this presentation, attendees will understand the types of fatal occupational injuries, the demographics of the victims, and the trends in fatalities nationwide.

This presentation will impact the forensic science community by increasing awareness of workplace fatalities, detailing the various injuries, associated occupations, and personal risk factors.

Although worker injury and fatalities have decreased since adoption of the Occupational Safety and Health Act (OSHA) in 1970, they remain an important safety issue.[1] In 2010, 4,690 workers were killed at work, which averages nearly 13 deaths per day.[2] The United States Bureau of Labor Statistics calculates the number of fatal occupational injuries by detailed event or exposure, with transportation incidents being the number one cause (40%) of work-related fatalities nationwide. Other major types of fatal injury include assaults and violent acts (18%), contact with objects and equipment (16%), falls (14%), exposure to harmful substances or environments (9%), and fires and explosions (4%).[3]

Presented will be the case of a 27-year-old white male who was found unresponsive in a chemical dryer at an industrial plant where he and his coworkers had been pressure washing the containers. He had entered the container in order to rescue a coworker who had lost consciousness. After lifting his coworker to safety, he was overwhelmed with the chemical fumes and became unresponsive. Resuscitation efforts were unsuccessful. Toxicological analysis did not reveal an etiology, but possible exposures included cyanuric chloride, cyanuric acid, or high concentrations of nitrogen. As a result of this incident, the company was fined by OSHA for serious violations including failure to post danger signs, lack of a ventilation procedure in confined spaces during cleaning, and failure to develop emergency rescue procedures. [4]

Discussed will be several notable trends in the last several decades, both in the types of injuries and the occupations associated with the fatalities. These include decreases in workplace homicide and work-related highway incidents, but increases in fatalities from fires and explosions, exposure to harmful substances or environments, and falls. The occupation with the highest number of fatalities is construction, but the highest rate of fatalities per 100,000 full-time equivalent workers is in agriculture, forestry, fishing, and hunting.[3]

Additionally, other personal risk factors such as age, gender, chronic disease, smoking, and alcohol and drug use have been implicated in workforce health and safety.[5] Examined will be the differences in types of workplace fatalities in male and female workers, as well as the disproportionate number of fatal injuries involving men.[3] Other significant populations considered include workers over the age of 55 and workers born outside of the United Sates.

Explored using this case example will be the role of the forensic pathologist in the investigation of workplace deaths as a joint effort with police, the district attorney, and OSHA.[6] The function of OSHA, including the standards set and penalty for violation of these standards, will also be reviewed. Finally discussed will be the future of occupational safety and the current improvements brought about by such incidents.

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Workplace, Fatalities, Asphyxia

G99 Exotic Aortic Dissection Treated With Formalin: Macroscopic and Microscopic Findings — A Case Report

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After attending this presentation, attendees will have a better understanding of macroscopic and microscopic aspects of acute aortic dissection.

This presentation will impact the forensic science community by providing an example of sudden death with poisoning suspicion from which a medicolegal autopsy was performed 15 days after death and after embalming.

Aortic diseases contribute to the high overall cardio-vascular mortality. The prevalence of aortic dissection is 0.5 to 2.95/100,000/yr. The diagnosis of aortic dissection has been missed in up to 38% of patients on initial evaluation. In up to 28% of patients, the diagnosis has been first established at the postmortem examination. The mechanisms of disease have been well established. Aortic dissection can result from intimal rupture followed by cleavage formation and propagation of the dissection into the media, or from intramural hemorrhage and hematoma formation in the media subsequently followed by perforation of the intima. Men are more frequently affected and chronic systemic hypertension is the most common predisposing factor. Cocaine use has been suggested as a possible cause of aortic dissection. The classifications classically used were Stanford and De Bakey classifications, which respectively subdivided aortic dissection in two (A and B) and three (I, II and III) groups according to the dissection location (ascending or descending aorta). Svensson *et al*. proposed a new classification in five classes in 1999 according to the intramural hemorrhage, intramural hematoma, aortic ulcers, or iatrogenic and traumatic dissection. Reported is a case of unexpected death of a man in a suspicious context of poisoning and discuss the autopsy and histopathological findings.

A 58-year-old French man died suddenly in Madagascar. He had lived in Madagascar for six years and, according to the police officers in charge of investigation, his wife could have poisoned him. His past medical history was not known but he had consulted a doctor who had performed an Electrocardiogram (ECG) and laboratory tests a few times before the death. The ECG showed a sinusal rhythm with no abnormality and the results of the laboratory tests were in normal range. The public prosecutor of Toulouse, France, ordered a medicolegal autopsy. The body had been embalmed and placed in a hermetic coffin during the flight from Madagascar to Toulouse. Two board-certified forensic pathologists performed an autopsy fifteen days after the death. All three body cavities (cranium, thorax, and

abdomen) were examined. Pathological examination was performed after fixation in 10% formalin.

The body was of strong build, length 170cm and weight 85kg. Two bleus hematomas were visible on the anterior face of the left forearm and on the posterior face of the left hand. No other external traumatic injuries were noted.

All the organs were well conserved because of formalin conservation. Examination of the thoracic cavity revealed a haemopericardium with 214cc of blood clot with formalin. The heart was enlarged and heavy (566g) with the appearance of dilated cardiomyopathy. An important aortic dissection was noted from the aortic root to the right and left common iliac artery. The intimal tear was found on the ascending aorta under the aortic arch. A widely intramural hemorrhage was also noted at the right coronary artery attesting to a retrograde dissection. These findings corresponded to a Stanford class A or De Bakey type I.

Microscopic studies confirmed the existence of aortic dissection with a widely intramural hemorrhage. The large false lumen affected the ascending aorta, aortic arch, right common carotid artery and subclavian artery, descending aorta, and both common iliac arteries. Histopathological examination also confirmed the existence of retrograde dissection to the right coronary artery. The dissection reduced 90% of the true lumen of the right coronary artery due to the compressive false lumen. The intimal tear was located 2cm above the aortic cusps. Examination of the kidneys revealed nephroangiosclerosis lesions due to a medical history of chronic systemic hypertension. Microscopic studies found signs of prolonged circulatory failure like acute cardiac liver, renal ischemia, and a fibrin clot formation. The forensic experts concluded that the cardiac tamponade was sufficient to explain the cardiovascular arrest and death of the man.

Cardiovascular diseases are the major cause of death in the majority of the developed countries and in many developing countries. Furthermore, due to the high mortality of aortic dissection in the acute stage, the survival rate is very low. Up to 20% of patients die before reaching the hospital. Thus, aortic dissection was the subject of many of necropsy studies. In a series of 150 postmortem tomographic examinations of non-traumatic death, Shiotani *et al*. noted 23 cases of aortic dissection. At the institute of Legal Medicine of the Hanover Medical School, 30 cases of aortic dissecting aneurysms were examined histologically between 2006 and 2009. The cause of death was a rupture into the pericardial sac in 28 cases (93%). The forensic experts performed an autopsy fifteen days after death and after embalming. They found the cause of death and excluded the suspicion of homicide. Some authors studied cases of autopsies realized with delay. Karger *et al*. retrospectively analyzed 155 forensic exhumations. In this study, postmortem interval ranged from eight days to eight years. Major discrepancies between cause of death as stated on death certificate and as diagnosed after autopsy existed in 57 cases (37%). The large majority of exhumation autopsies were successful and the cause of death was clearly determined in 66.5% of cases. One case was reported of an autopsy realized nine days after the death and after burial and exhumation. The circumstances of death were unclear but, at the autopsy, forensic experts noted a massive hemoperitoneum secondary to hepatic artery rupture due to infectious arteritis. Classically, aortic dissection occurs in individuals with hypertension and individuals with genetic disorders of collagen formation such as Marfan's and Ehlers-Danlos syndrome. Men are more frequently affected, and the peak age for the occurrence of proximal dissection is between 50 and 55 years of age. Many studies have been reported of aortic dissection associated with cocaine or methamphetamine use. Cases of dissecting aneurysm or aneurysm rupture have also been reported in pregnancy and the postpartum period. In this case, the toxicological analyses were not performed, but according to sex, age, and nephroangiosclerosis, it was a classic case of sudden natural death in a 58-year-old man with cardiovascular risk factors. The medicolegal autopsy was necessary to find the precise cause of death and exclude homicide. Obviously, in cases where the cause of death is unclear, its determination by autopsy even some time after the death, can remove suspicion and allow the family of the deceased to mourn.

Aortic Dissection, Embalming, Medicolegal Autopsy

G100 Effect of Concealment on Necrophagous Flies Access

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After attending this presentation, attendees will understand how different combinations of accessibility and odor diffusion affect the access of bait by calliphorids necrophagous flies. Such results are needed in forensic entomology to estimate the Pre-Appearance Interval (PAI: delay before first insect arrival), especially regarding indoor cases.

This presentation will impact the forensic science community by providing, for the first time, data on flies' abundance and PAI depending on odor diffusion and accessibility.

Forensic entomology is used to estimate the age of the insects sampled on cadavers, and subsequently to estimate the time of death. However, flies do not always colonize corpses immediately after death. If the corpse is difficult to reach, especially in the case of wrapping, concealment, or burying, access by necrophagous insects may be delayed. Such a delay in insect arrival (PAI) is also observed with bodies discovered inside dwellings or vehicles.

It may seem obvious that the less accessible a corpse is, the later and fewer flies there will be; however, no known detailed experimental evidences have been obtained. Furthermore, a delayed/altered insect access on concealed or indoor corpses could actually result from two different processes: first, the difficulty for the insects to detect a corpse due to potentially low odor diffusion, and, second, the inherent difficulty in accessing concealed corpses. Thus, two different parameters overlap in insect "accessibility," the escape of gases and the surface allowing insects to enter. It was hypothesized that these two parameters both influence the colonization process, and therefore, must be considered together.

Two possible consequences of the low accessibility of a corpse could be: (1) an increase in the pre-appearance interval (i.e., the time before first insect arrival); and, (2) a decrease in the total number of flies accessing the corpse. Both can impact the decomposition timeline and, in a forensic context, the PMI estimation. To answer this question, experiments were performed under controlled (laboratory) and field conditions. Baited traps were used to test how different combinations of accessibility and odor diffusion affect the access of bait by calliphorids necrophagous flies. Although such experimental designs were not directly connected to "real" forensic cases, they covered a large range of possible scenarios and thus bring general information on the behavior of necrophagous flies.

During laboratory experiments, 30 *Lucilia sericata* gravid females were kept in a cupboard with one of the traps. The most efficient trap caught a mean of 24.6±3.4 flies per run. The less efficient one had the largest odor diffusion surface (99cm²) combined with the lowest accessibility (one 1cm² entrance hole). It caught a mean of 5±3.7 flies per run, and significantly differed from the other. Overall, the results indicate that: (1) *Lucilia sericata* can enter in barely accessible traps; and, (2) odor diffusion and accessibility both affect the number of flies entering the traps.

Field experiments were performed with the same trap designs. For these experiments, all the traps were placed together outdoors in the same place and at the same time. The trap with the larger entrance (ten 9cm² holes) caught the most flies (55.6% to 99.4% of the total number of flies caught during a single run). On the contrary, only a few flies entered the other traps. None of these traps exceeded 29% of the total number of flies caught during a single run. A delayed access of the less open (odor diffusion and accessibility) traps was finally noted. Major conclusions of field experiments are: (1) traps

with low accessibility took longer to be accessed by flies; (2) larger odour diffusion surfaces increased fly attraction; and, (3) flies more readily accessed traps through larger holes than through an equivalent surface area made up of smaller holes.

In a forensic-entomology context, results suggest that both accessibility and odor effusion should be considered together to assess the possible time that might elapse between death and first arrival by flies.

Entomology, PMI Estimation, Indoor Cases

G101 Traumatic Renal Artery Rupture Following a Fall: A Fatal Occupational Accident

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After attending this presentation, attendees will understand the importance of full forensic investigations in establishing the manner of death in occupational deaths. Circumstantial data and autopsy findings, together with a detailed workplace investigation, also are fundamental for the identification of legal responsibilities.

This presentation will impact the forensic science community by emphasizing the fact that, in some circumstances, the forensics have to take into account the traumatic involvement of vascular structures, even without external major injuries. From this perspective, the autopsy is crucial, mainly if death occurred at the time of the accident or immediately after and other causes (natural death, drug or alcohol abuse) must be evaluated.

Presented is a case of an occupational death due to rupture of the left renal artery in closed thoracic-abdominal trauma, after a fall from a height. Blunt renal artery injury is a relatively rare finding. The literature demonstrates an incidence of 0.08% of all blunt abdominal traumas, occurring in 1% to 4% of patients with renal injury. The most common cause is motor vehicle accidents; other causes include a direct blow to the chest or abdomen and a fall from a height. All of these mechanisms result in sudden deceleration or crush injuries, affecting the renal parenchyma or the vascular pedicle. Moreover, a traumatic ruptured renal artery, without grossly demonstrable kidney damage, is also unusual.

In the case presented, a 47-year-old man accidentally fell from a height of 2.5 meters while he was working on a scaffold. The worker was taken to the local hospital, but he remained unconsciousness and unresponsive. A rounded bruise of the left thoracic wall was noticed. At echocardiography, a hematoma was observed at the upper left abdominal quadrant. The patient was intubated and, despite the cardio-pulmonary resuscitation, he died 30 minutes later. With the goal of better understanding the dynamics of the event, a medicolegal autopsy and crime scene investigation was performed.

The shirt he wore showed a soft rounded mark on the left side of the chest. The external examination of the body showed only a rounded bruise of seven centimeters in diameter on the left hypochondrium. At autopsy, fractures of eight ribs were found (the majority to the left), with perilesional hemorrhages with nearly 100cc of fresh blood in pleural cavities. The abdominal cavity displayed two litres of fresh blood and clots. A complete rupture of the left renal artery was found at one centimeter from the origin from the abdominal aorta. No alcohol or drugs of abuse were found in the fluids collected at autopsy. The cause of death was identified as hemorrhagic shock due to the laceration of the left renal artery.

Frames of the surveillance cameras were acquired, showing the man sliding off the scaffold (made of metallic beams and wooden traverses) and falling on the left side of the body, without displaying where he landed.

At the workplace investigation, a footprint on the external side of the scaffold was found, where the victim was working; the footprint matched shoes worn by the worker. On the grassy ground below, was a metallic pedestrian gate with a 1-meter-high hinge post placed just below the side of the scaffold, 2.52 meters from the footprint. The final reconstruction of the fatal accident concluded the man had fallen onto the gate hinge post, hitting the left side of the thorax; the blunt injury caused the rib fractures and the laceration of the left renal artery without any other injury of internal organs.

This report of isolated renal artery rupture represents an unusual finding, extremely rare following blunt abdominal trauma, and accounts for less than 0.1% of all trauma patients. Moreover, traumatic renovascular injuries occur most often in multiple injury patients. In the presented case, the preliminary reconstruction of the work-related fatality was not clear before the autopsy as there were no major external lesions. Therefore, the autopsy detected the cause of the death, allowing the real traumatic mechanism to be traced.

Traumatic Artery, Occupational Fatality, Workplace Investigation

G102 Forensic Pathology Considerations in the Transgender Population

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After attending this presentation, attendees will be able to describe the possibilities of cause/manner of death in the transgender population based on their reported marginalization and lifestyle risk factors. Attendees will be aware of the increased proportion of viral disease, and the importance of variable medical and/or surgical transition features.

This presentation will impact the forensic science community by increasing awareness of the challenges of postmortem examination of transgender individuals.

Transgender (TG) refers to individuals who express their gender differently than expected by society based on their natal sexual anatomy, with a range of gender variant identities. A minority have undergone “full” surgical sexual transition. Their lifestyle risk factors, as reported primarily in a large study with greater than 7,000 respondents, apply to all TGs. It is important to recognize persons who identified as transgender as there are likely to be a number of possibilities and factors contributing to death. Forensic pathologists should be aware that information about sexual identity may not be specifically sought or provided, and changes may not be obvious upon examination.

There are only a few somewhat sensational case reports on TGs in the forensic literature. Studies are logistically difficult, and there are few cohort studies.

TGs face significant barriers to medical care. For those few with insurance, it is unlikely to cover expanded recommendations for health care, or the costs of medical/surgical transgender affirmation interventions. Disturbingly, in medical settings, 19% have been refused care, 28% reported harassment, 2% have been the recipients of violence, and 50% reported that their physicians lacked treatment knowledge. Many eschew health care, and natural conditions may be neglected.

Routine health care maintenance is not simple, because they require surveillance for conditions affecting both the natal and surgically-created sex organs, as well as conditions secondary to hormone use (the effects of which remain unclear). Up to 50% of TGs have used “illegitimate” source hormones. Some inject foreign substances for cosmesis, with possible systemic complications. Self-mutilation is common.

Rates of substance abuse (alcohol, drugs, tobacco) and psychiatric illness are significantly greater among TGs.

Almost all report bias, discrimination, “transphobia,” and frequently assault. The homicide rate is unknown, although there have been prominent cases in the media (and in film). The U.S. Hate Crimes Prevention Act was expanded to include a victim’s actual or perceived gender or orientation.

Analysis was performed on 33 TG individuals who presented to DOFM, Glebe, between 1993 and the present. Although this number

is too small for formal epidemiological analysis, descriptive examination shows features that generally reflect the reported lifestyle risk factors.

Where known, the risk factor frequencies are cited in narrative or brackets. Fifteen percent of the deceased group had "complete" surgical transition (penectomy +/- vaginoplasty), representing 15% of the MTF group (20%) and none of the FTM group (2%). Thirty-nine percent of the deaths were due to alcohol and/or drug toxicity; 26% reported use to "cope," up to 36% in individuals involved in "underground economies" (i.e., prostitution). Fifty-one percent of the deceased group had a history of substance abuse. The suicide rate was 18% (with an astounding 41% of the surveyed group reporting suicide attempts, 25X that of the general population). Four of the group (12%) were infected with HIV, all having been routinely tested; the expected rate is 4X the national US. average, or 3.76%. Twenty-seven percent were infected with hepatitis C virus. One death, of the 27% natural deaths, was directly due to HIV disease. Seventy-two percent of the deaths occurred under the age of 50. None were homicides. The rate of unnatural death was slightly greater than 75%.

One death depicts the unusual situation of a late complication of TG surgery as a possible contributing factor to death. The deceased had thromboembolic disease on a background of unknown current estrogen use, colorectal carcinoma *in situ,* and chronic ethanolism; there was also acute and chronic cystitis/prostatitis, the latter almost certainly due to the specific anatomy of the genitalia. This case illustrates the need to identify and examine every TG case in terms of their specifically altered anatomy and physiology, as well as an awareness of their substantial lifestyle risk factors.

Transgender, Lifestyle Risks, Postmortem

G103 Exogenous Lipoid Pneumonia: An Uncommon Incidental Autopsy Finding

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After attending this presentation, attendees will learn about lipoid pneumonia, a rare condition that may be incidentally found during postmortem investigation. Knowledge of the characteristics of this condition may allow revealing various risk factors that are helpful in the determination of cause of death.

This presentation will impact the forensic science community by highlighting an uncommon finding during autopsy. Basic knowledge of the characteristics of lipoid pneumonia may be helpful in the determination of cause of death.

Postmortem investigation often leads to incidentally revealing various conditions, which may or may not have played a part in the death of an individual. Lipoid pneumonia is a rare disease that is not necessarily involved in the occurrence of death, even as a contributory factor, but knowledge of the predisposing factors may be helpful in the determination of the cause of death of an individual.

Reported was a case of a 32-year-old woman who was found dead in her bed by her husband. According to him, she had taken methadone and cannabis the day before. She had a long history of drug abuse and was treated with buprenorphine in a drug addiction treatment center for six months. She also suffered from anorexia during her teen years, and had a history of suicide attempts.

External examination showed no traumatic injuries and no signs of intraveinous injection. At autopsy, a diffuse pulmonary edema (left lung: 816g; right lung: 998g) and congestion of lungs, brain, liver, and spleen were noted. The autopsy was otherwise unremarkable.

Toxicology revealed the presence of methadone (0.77µg/mL) and EDDP, its major metabolite (0.17µg/mL), buprenorphine (1.4ng/mL), amitriptyline (0.24µg/mL), olanzapine (0.20µg/mL), tropatepine (0.01µg/mL), nitrazepam (<0.01µg/mL), clonazepam (<0.01µg/mL) and 7-aminoclonazepam (0.06µg/mL), and zopiclone and tetrahydrocannabinolic acid (3.6ng/mL).

Miscroscopic examination of the lungs showed a diffuse interstitial fibrosis, with multiple intra-alveolar and interstitial foamy macrophages, and extracellular fat droplets with multiple polynuclear giant cells. Areas of subpleural emphysema were also seen. The brain, heart, and other organs were unremarkable. A diagnosis of lipoid pneumonia was made.

The medical record of this woman was studied and it was learned that, one year before she died, she had been hospitalized in a pneumology department. She was complaining of a moderate dyspnea and weight loss. A chest X-ray showed an opacity of the right lung. A thoracic CT scan revealed multiple ground-glass opacities and pulmonary emphysema. A bronchoalveolar lavage was performed, showing the presence of multiple macrophages and lipidosis. A diagnosis of exogenous lipoid pneumonia was made. The main risk factor was found to be a chronic intranasal use of various susbtances.

Lipoid pneumonia is an uncommon entity with the characteristic radiograph features and histologic findings of alveoli filled with vacuolated, lipid-laden histiocytes. It can be either exogenous or endogenous in cause based on the source of the lipid. Exogenous lipoid pneumonia usually results from aspiration or inhalation of fat-like material, such as mineral oil or petroleum-based lubricants and decongestants, resulting in pulmonary inflammatory reactions. The clinical findings may vary from the absence of symptoms to chronic cough, fever, hemoptysis, and dyspnea. Abnormalities are usually seen on chest X-rays, as an area of homogenous dense consolidation, or presence of nodules. CT scan can reveal areas of fat attenuation and ground-glass opacities. Endogenous lipoid pneumonia is an obstructive pneumonitis. It can be associated with non-small lung cancers but also can occur as a manifestation of infection and other diseases. It can also appear with fat emboli, pulmonary alveolar proteinosis, and lipid storage disorders. Polarized light microscopy after staining with sulfuric acid and acetic acid usually reveals cholesterol crystals, a finding diagnostic of endogenous lipoid pneumonia.

In this case, death was attributed to acute poly drug intoxication. As microscopic examination had revealed severe pulmonary lesions, lipoid pneumonia and pulmonary emphysema were considered as conditions contributing to the cause of death.

Forensic Pathology, Lipoid Pneumonia, Drug Intoxication

G104 A Comparison Study on the Performance of STR Typing Kits With Improved Buffer Systems

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After attending this presentation, attendees will gain information regarding STR typing kits with improved buffer systems which offer better tolerance to inhibitors commonly encountered in forensic casework samples.

This presentation will impact the forensic science community by discussing how studies showed the improved buffer systems in these newly developed kits are promising and could be employed for DNA typing of challenging samples.

DNA fingerprinting, since its introduction in the 1980s, has frequently been employed by forensic scientists to assist in the identification of individuals. Analysis of Short Tandem Repeats (STR) using Polymerase Chain Reaction (PCR) technique, which enables minute quantities of DNA to be detected, has replaced the Restricted Fragment Length Analysis (RFLP) technique since the mid 1990s. Though powerful in assisting forensic investigations, DNA fingerprinting is not straightforward.

One of the challenges of DNA fingerprinting is that, in many forensic cases, the DNA samples are far from pristine. Amplification of compromised samples can result in poor or no genotyping results, whereas amplification in the presence of inhibitors can even lead to loss in signals. The first issue has been overcome by the introduction of the analysis of STR with shortened amplicons, which are optimized for genotyping degraded samples. Meanwhile, several new STR typing kits with modified buffer systems claiming improved tolerance to inhibitors commonly encountered in forensic casework samples have been introduced to the market in recent years.

To study if these newly developed kits will perform better than the one currently being used in the laboratory (which has been on the market since 2001), three newly developed kits, A, B, and C from three companies,were selected and their performance compared in the following aspects: (1) detection sensitivity; (2) PHR, (3) stutter ratio; (4) intra-/inter-color balance; (5) concordance of typing results; (6) tolerance to inhibition by hematin and humic acid; and, (7) performance for challenging samples. A total of 35 samples were employed in this study, and were amplified according to the conditions as suggested by the respective manufacturers. The amplified products were injected into a genetic analyzer commonly used in forensic DNA analysis, and the data were analyzed with the software provided.

In summary, as compared to the STR kit currently used in the laboratory, all three tested kits demonstrated improved detection sensitivity with comparable PHR in various amounts of input DNA (0.125, 0.25, 0.5, and 1ng). They exhibited better tolerance to inhibition by hematin and humic acid, and showed obvious improvement in the analysis of challenging samples. In addition, full concordance was observed in a total of 375 STR allele calls in the analyses. Among the three kits, kit A displayed the best intra-/inter-color balance, the highest tolerance to inhibition by hematin and humic acid, and the greatest improvement in the analysis of challenging samples. It is noteworthy to mention that, although kits B and C (with increased PCR cycle numbers) showed greater enhancement in sensitivity, the stutter ratios of these kits were also elevated as compared with kit A. To conclude, studies showed that with the modified buffer systems, the performance of all these newly developed kits are comparable and even better than the currently used STR typing kit. In addition, they demonstrated improved performance in the analysis of challenging samples. Therefore, all are suitable candidates for the selection of the next generation STR typing kit in the laboratory.

STR, Inhibition, Tolerance

G105 Preconception Diagnostic Approach: Case Report

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After attending this presentation, attendees will see the results of the preconception diagnostic approach that can lead to death and the importance of detailed examination.

This presentation will impact the forensic science community by increasing awareness that preconception diagnosis approaches cause negativities at patient's treatment and follow up and the best approach is detailed examination and laboratory investigation.

Sudden unexpected deaths constitute a large part of forensic medicine's daily work and are mostly caused by cardiovascular system disorders. It was noted that acute myocardial infarction and coronary artery disease are mostly seen, but sudden deaths due to aortic dissection and rupture are less seen.[1]

Aortic dissection occurs by the rupturing of intima layer and, passing blood to the middle and two-thirds part of the media. This situation occurs mostly at the ascending aorta and mostly has a transverse course. Aortic dissection is a life-threating disease which is characterized by sudden chest and/or waist pain. The disease is seen most commonly in men.[2] Compensation mechanisms are not enough because aortic rupture cases are too severe and progress very rapidly. It was seen that of the 85.4% of cases located in the thorax, aneurysm was at the ascending aorta with 70% of them at the opening to the pericardium.[3] Despite the improvements of noninvasive diagnostic methods, acute aortic dissection's mortality is still high. Delaying the diagnosis affects hourly mortality by an increase of 1%. A rapid and correct diagnosis can place the mortality rate under 50%. Mortality rate of the disease was 1% per hour in 24 hours, especially at dissections, on the ascending aorta. This rate rises to 75% at the end of second week. When considered from this point, early diagnosis is an important factor which positively affects the prognosis.[4]

Introduction: The most important usage area of the pesticides is in agriculture as pest control. In 1985, organochlorine pesticide usage was forbidden except endosulfan and toxaphene.[5] Endosulfan (a neurotoxin) poisoning may cause neurologic symptoms like tonic clonic seizures, tremor, headache, dizziness, ataxia. The treatment approach is symptomatic, seizures can be controlled by benzodiazepine and if needed by phenobarbital.[6]

Case: It was detected that a 25-year-old male fainted while spraying his garden and was brought to hospital. He was unconscious, his pupils were dilated, and there was no light reflex. He was intubated due to shallow respiration and, right after cardiac arrest occured, he was resuscitated with cardiac massage and medical theraphy. It was thought that he was poisoned by endosulphane pesticide, so gastric lavage was done by using nasogastric tube. He was comatose and continuously having seizures. Benzodiazepine was used at his treatment. During his medical theraphy he was arrested and atropinized; defibrillation was performed, but he expired, not responding to resuscitation. At his autopsy, heart was weighted as 380g, 400cc bloody fluid was detected at his pericardial sac, there was 2cm dissection at his aortic knob, he was died because of pericardial tamponade which caused by aortic dissection. At his toxicological report, 143ng/ml active substance was found in his blood which belongs to benzodiazepine family and an antidepressive drug (diazepam).

Conclusion: There may be a few possibilities which caused undetected endosulfan level at his toxicology report. The first possibility is atropine which was used at treatment and not reported pralidoxime smooth the excretion of endosulfan. Another alternative is incorrect laboratory testing results. And the last and most important alternative is misdiagnosis. It was important that physicians consider endosulfan poisoning at the differential diagnosis instead of cardiac events for a patient who had central nervous system toxicity symptoms and signs with undefined etiology and who came from countryside. But this hospital and other healthcare organizations don't have required conditions for this kind of considerations. This is a dead end and also every condition of the country about this area must be used in situations like that.

Noted in the physicians logs or notes; mistakes were made because of misdiagnosis due to general assessments. Because of this, the best approach is to make the decision after a detailed examination and appropriate laboratory investigations.[7]

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Diagnose, Autopsy, Aneurysm

G106 A Fatal Case of Human Herpes Virus 6 Fulminant Pneumonia in a Young Immunocompetent Woman

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After attending this presentation, attendees will understand the importance of a complete forensic approach, through autopsy, histological, and virological examinations, in an uncommon case of lethal fulminant hemorrhagic pneumonia in a young immunocompetent woman and in determining Human Herpes Virus 6 (HHV 6) infection as the cause of death.

This presentation will impact the forensic science community by showing that acute HHV6 infections can occur in adults suffering a variety of syndromes, from non-specific to severe in both immunosuppressed and healthy patients.

Primary HHV6 infection causes *exanthema subitum*, a common febrile disease of infancy, whose clinical course is generally benign and self-limited. HHV6 is usually acquired at a young age and remains latent in the salivary glands and central nervous system of healthy subjects. Reactivation of latent HHV6 is common and can cause, in immunocompromised hosts, severe complications including encephalitis/encephalopathy, pneumonitis, hepatitis, thrombocytopenia, hemophagocytic syndrome, and myocarditis.

Pneumonia associated with HHV6 infection is interstitial, and it has been repeatedly reported in the literature in patients after allogenic stem cell transplant or with HIV infection. The clinical findings vary from mild to severe, requiring mechanical respiratory support attributable to Acute Respiratory Distress Syndrome (ARDS).

The case of a 27-year-old woman who was admitted to the emergency room complaining of cough, dyspnea, hemoptysis, and fever was reported. The clinical history of the patient was silent for any disease. A chest CT scan showed multiple foci of pneumonia of the right lung, with epato-splenomegaly, and mediastinal lymphadenomegaly. The blood exams showed severe decreases of leukocytes, platelets, and lymphocytes, and pseudo Pelger-Huet anomaly of neutrophils at peripheral blood smear. As the patient worsened, she was intubated with blood leaking from the airways. Despite medical resuscitation, the woman died eight hours after the admission. Due to the lack of previous medical history, a medicolegal autopsy was performed.

The external examination of the body was completely negative. The autopsy revealed hemorrhagic pleural effusion (1200cc total), pulmonary edema with hemorrhage, and epato-splenomegaly.

The histological examination revealed bilateral pneumonia with interstitial lymphocytic infiltrates and diffuse alveolar damage (ARDS-hyaline membranes). No alcohol or drugs of abuse were found in the blood and urine collected at autopsy.

Microbiological analysis was negative. Virological examinations of the pleural effusion and blood showed HHV6 pneumonia with low levels of Human Herpes Virus 7 (HHV7) and Epstein-Barr Virus (EBV), demonstrative for a co-infection.

The cause of death was HHV6—haemorrhagic pneumonia (with co-infection of HHV7 and EBV) that evolved into ARDS.

Some studies have identified co-infections with HHV6 and other viruses in a surprising number of HHV6-associated pneumonias. In fact, HHV6 and HHV7 are ubiquitous in the adult population and can reactivate periodically and cause several manifestations reported to occur at high frequency when the functions of cell immunity are impaired.

In this case, the young patient was not immunodepressed, without other pathologies, and developed a fatal pulmonary failure attributable to HHV6 pneumonia, evolving into ARDS. According to the literature, ARDS caused by HHV6 is extremely rare, and is described mainly in immunodeficient persons.

The evidence of a detectable viraemia has a key role in the diagnosis of the infection, which has to be identified and treated with specific antiviral drugs, as soon as possible. Nevertheless, virological exams, based on the quantification of viral load in bodily fluids, are available but need to be improved and standardized. Although no systematic evaluation of treatment regimens is available, coincidental administration of antiviral drugs can not result in clinical improvement.

Autopsy, Human Herpes Virus 6, Pneumonia

G107 Deaths in Custody: A Northern Portuguese Analysis From 2000–2010

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After attending this presentation, attendees will have a better and clearer perception of the northern Portuguese situation on the topic of deaths in custody from a medicolegal perspective.

This presentation will impact the forensic science community by presenting the first Portuguese retrospective study of deaths in custody from a medicolegal point of view, analyzing important issues, such as demonstrating the cause of death, as well as circumstances and manner of death.

Deaths occurring while individuals are in custody, given their peculiarities, are quite tragic and distressing for the individuals' families and friends, police enforcement agencies, and the institutions where they occur. The same applies in a broader perspective to society, especially with those cases in which death happens in a sudden, unexpected, and violent manner. The suspicion of excessive force or violence against the arrested decedent on behalf of the police agents within jails and penitentiaries is always the subject of extensive media coverage, often even without true knowledge of the real facts. Thus, "deaths in custody" are of undeniable forensic interest, considering the demonstration of the cause of death, as well as the circumstances and manner of death. In Portugal, there are very few studies on this matter and none of them from a medicolegal point of view since the approach to this subject has been quite infrequent. Moreover, the studies published in the international reference forensic

literature relate to economic and socio-cultural realities which, in most cases, are rather different from the Portuguese one.

This study intends to analyze and characterize, from a medicolegal perspective, all deaths which occurred in the north of Portugal between 2000 and 2010 as a result of police vs. citizen interaction, when the decedent was under the responsibility of any police enforcement agency or security service. This study was based on data obtained from different police enforcement agencies and security services, autopsy reports, and criminal investigation process files. A total of 228 individual cases of prisoners in 13 different penitentiaries were analyzed, as well as 130 autopsy reports and 10 criminal investigation process files in different courts of law.

During the timeframe covered by this study, 237 fatalities were found that met the criteria for "death in custody." Of those 237 deaths, 55.3% underwent a forensic autopsy. "Deaths in custody" occurred in 225 cases under the jurisdiction of penitentiaries and correctional facilities, in seven cases under the jurisdiction of the PSP (Public Security Police), and in five cases under GNR (National Republican Guard) jurisdiction. The most common profile in these fatalities, according to the collected data, corresponds to male individual (98.3%), single (56.5%), aged between 25 and 44 (61.1%), with low level education (36.3%), resident in the Porto District (67.5%), and suspected of or convicted for crimes against personal or public property (31.6%). From a medicolegal etiology point of view, death from natural cause was the more frequent etiology (59.5%), followed by suicide (25.7%), accidental causes (8.9%), and homicide (4.2%). HIV infection/AIDS were responsible for 17.7% of the death cases studied. Hanging was the most frequently used method for suicide in jails (24.5%). There were ten homicides, two of which resulted from physical assaults between inmates and eight resulted from the police enforcement agent's action at the moment of approach/detention.

Death in Custody, Police Enforcement, Cause of Death

G108 Correlation Between Thyroid Disease and Sudden Thromboembolic Death: Case Report

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After attending this presentation, attendees will understand how thyroid disease can be related to sudden thromboembolic death in young people.

This presentation will impact the forensic science community by underlining the possibility of a sudden death caused by thromboembolism, consequent to thyroid disease.

Venous thrombosis is the third most common cardiovascular disease after myocardial infarction and stroke. The incidence rates for VT (1 – 3/1.000) vary between person/years.[1-3] The case fatality rate of profound venous thrombosis, mainly due to massive pulmonary embolism, varies from 1% in young patients to 10% in older patients. Often the diagnosis of pulmonary thromboembolism is discovered during autopsy.[4,5] In the United States, pulmonary embolism causes about 50,000 deaths per year.[6] In general, the thromboembolism determined a sudden death, especially in young people, and it is not always preceded by prodromal symptoms.

The literature suggests a moderate association between hyperthyroidism and acute venous thrombosis. In particular, the role of thyrotoxicosis is a common disorder with an incidence between 0.5% to 2.5% in the world. It causes large effects on the heart, reversible changes such as: increased prothrombotic VIII factor and von Willebrand factor; an increase of clots most resistant to fibrinolysis.[7-11]

The purpose of this study is to detect a possible association between thyroid disease and sudden thromboembolic death.

The case study examined a 41-year-old woman, mother of two children, found dead in the bedroom of her lover. Investigation at the scene found that the corpse was naked on the bed. The police reported the woman had had sexual relations with the man. From the investigation interview her doctor did not suggest any disease and the woman had been in good health. The external inspection of the cadaver did not detect external signs of a struggle or other injuries. The TCMS postmortem showed an alteration of TC densitometry in correspondence of the right ventricle. The survey autopsy showed a massive thrombus-embolism without other evident internal signs. Histological examination revealed a pituitary adenoma with lymphocytic thyroiditis of Hashimoto disease. The histological finding, the absence of other diseases or other predisposing risk factors for thromboembolism were allowed to correlate the genesis of thromboembolic sudden death with thyroid disease, which the victim had.

The study has value for the scientific community because it focuses attention on the problem of sudden deaths in young people. In particular, it highlights the high risk of mortality, as demonstrated in the literature, for people with thyroid disease in the genesis of sudden deaths of thromboembolic death. The prevention of these diseases and the appropriate therapy in patients would help to avoid such events.

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Sudden Death, Pulmonary Embolism, Thyroid Disease

G109 Varicose Vein Rupture: Crime Scene of Uncertain Non-Natural Death?

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After attending this presentation, attendees will be able to evaluate the possible consequences of varicose vein rupture and the importance of an adequate scene investigation to understand causes of death.

This presentation will impact the forensic science community by discussing varicose vein rupture and its mortal complications.

One of the most commonly reported chronic medical conditions is represented by venous disease, including varicose veins and Chronic Venous Insufficiency (CVI). It is also a substantial source of morbidity in the United States and the Western world. Varicosis is found in 15% – 50% of the population. Clinically relevant classification of varicophlebitis appears useful: stage one includes varicophlebitis without involvement of the respective junctional valve in the groin or at the knee and deep veins; while in stage two, the proximal part of the thrombus has reached the respective junctional valves of the long or short sapheneous vein; in stage three, it has entered the deep veins by means of these valves; in stage four, the thrombus migrates via insufficient perforating veins into the deep system. Several risk factors associated with the development of varicose veins and chronic venous insufficiency or both are older age, female gender, family history, obesity, and a standing occupation. Small Arteriovenous Communications (AVCs) are an important etiology of varicose veins and stasis ulcers of the lower extremities. Hemorrhage from ruptured varicose veins of the legs can occur spontaneously or after a minor trauma. More frequent complications of varicosis include peripheral edema of the ankles, skin ulcers, and varicose eczema. On the contrary, in most forensic practices fatal hemorrhage from rupture of varicosis is a rare event.

The goal of this study is to analyze the importance of inspection and on-site forensic investigations to determine the cause of death when the crime scene is uncertain.

One case of varicose vein rupture is reported. An 80-year-old man was found dead in the bedroom of his apartment. Traces of blood were found in the intire room. The body showed typical signs of death due to exsanguination. A large pool of blood was found on the floor. The scene analysis allowed for the evaluation of the presence of small spatters of blood on the back of the left foot. An external examination of the victim showed a circular lesion linked to subcutaneous arteriovenous anastomoses. Blood spatter investigations and circumstantial data allowed for the detection of the cause of the breaking of the ulcer. In fact, the sock worn by the victim presented a circular blood crust that served as a “cap” on the skin lesion (ulcer).

Analysis of the collected data showed that the elderly man, removing the sock, tore out the crust (cap) from the lesion when removing the sock. Other traumatic lesions were not found. In particular, blood stain pattern analysis was performed in order to understand the origin and the location of venous and/or arterial bleeding. However, blood projected from ruptured varicose veins of the lower limbs may also result in a similar pattern of projected, disseminated fine bloodstains.

In this case, the cause of the hemorrhage was a small lesion of the skin of the left lower leg of the victim, linked to a small arteriovenous communication. At first sight, fatal hemorrhage, with massive traces of blood, may associate the death scene to a crime, with focus primarily on a non-natural death. This case demonstrated the central role of crime scene investigation in these cases. Further, this study showed the fundamental role of prevention in cases of varicose vein disease through adequate treatments.

Hemorrhage, BPA, Varicose Vein

G110 Postmortem Multi-Slice Computer Tomography in the Evaluation of Single Gunshot Injuries

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After attending this presentation, attendees will understand the role of postmortem imaging in the evaluation of single gunshot injuries.

This presentation will impact the forensic science community by discussing the usefulness of Multi-Slice/Computer Tomography (MSCT) in the description of gunshot injuries in relation to entrance and exit wounds and bullet paths, as well as in 3D reconstructions.

Postmortem MSCT is a new approach in forensic pathology for helping investigations. Gunshot injuries are one of the foremost fields of postmortem forensic radiology. MSCT is performed to locate the projectile, to identify entrance and exit wounds, to detect bullets and bullet fragments in the body, to show the bullet course, and other, inflicted injuries. Also it is possible to do 3D reconstruction of the soft tissue and skeletal injuries.

The purpose of this study is to determine the role of postmortem multislice computer tomography in characterization of gunshot injury to reconstruct the site gunshot entrance wound and the direction of the bullet path.

In this study three cases of death due to single gunshot injury are reported. In the first case, an 80-year-old man was found dead in the bedroom of his apartment with a gun in the right hand. An external examination of the victim showed devastating head injuries. In the second case, an 81-year-old man was found dead in his apartment. He did not have any gun in his hand because it was removed by the police. In this case an external examination showed the anatomical site of gunshot injuries. In the third case, a 56-year-old man was found dead on the road near the center of the city. Postmortem radiological investigation (MSCT) and autopsy examination were performed in these three cases. The determination of entrance and exit wounds was reconstructed from the characteristic fracture pattern with inward or outward bevelling of the bone respectively.

In this study, MSCT showed an overvalued internal hemorrhage and a precise detection of skeletal injuries and skull fractures. Circumstantial data and autopsy investigation showed cause and modality of death. For this reason radiological approach is very important for reconstruction of gunshot injuries but it does not give any information about modality of death.

In some countries, the use of imaging techniques such as computerized tomography and magnetic resonance is becoming a routine procedure before the autopsy, or even, in some cases, investigations carried out in place of the autopsy. Other advantages of this investigation are: presenting clear and objective information to the court as forensic evidence, 3D documentation of the investigation, and quality assurance through the digital data filling and transfer. The documentation and analysis of postmortem reports identified by MSTC, a non-invasive method, will lead over time to qualitative improvements in forensic pathology investigations. The methodological operative combination between the two disciplines (radiology and forensic pathology) has begun to define common objectives, especially through the use of methods such as MSCT, which are:

- Determination of the cause and modality of death.
- Evaluation of vital signs depends on the presence of anatomical damaged structures.
- Developing a forensic reconstruction based on various reports.

Currently, MSTC is a method increasingly used for forensic purposes so as to have the potential to enhance today’s formal procedures. Apart from the accuracy and three-dimensionality, this method allows the corpse to be re-examined even after some time. In fact, it is believed that this virtual approach, non-invasive or minimally invasive, will improve forensic pathology tools in the near future.

Gunshot, Virtopsy, Autopsy

G111 Automated Reporting System Increases Referral and Donors

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The goal of this presentation is to alert medical examiners, coroners, and organ procurement agencies of the benefits of automated reporting of potiential donors to local organ procurement agencies. This system saves time and organs.

This presentation will impact the forensic science community by increasing organs for possible donation, complying with state statutes on reporting potential donors, and improving overall efficiency for medical examiners' and coroner's offices. This encourages improved data exchange between agencies which elevates the professional status of the forensic community.

As the number of accessible organs and tissues continues to remain insufficient to supply the current demand, deaths investigated by medical examiners' and coroners' offices may prove to be a significant source of viable organs and tissues; however, tighter budgets and increases in mandated reporting requirements have caused friction between many medical examiners'/coroners' offices and some organ procurement organizations. The need for collaboration and timely communication between these agencies is essential if each is to fulfill their responsibilities to the public and others who rely on their services. Although most states require medical examiners and coroners to report all deaths to the local organ procurement agency, many viable organs and tissues expire due to needless delays in notification, recovery, and procurement tasks. Although medical examiners' and coroners' offices have spent hundreds of thousands of dollars on "state of the art" case management systems, most are antiquated before they "work," and rarely do these systems allow (or are capable of) communication with other data systems. Reporting and information exchange regarding potential organ and tissue donors is time consuming, inefficient, and typically done—by telephone—daily.

The Washtenaw County medical examiner office in Ann Arbor, Michigan, along with Occupational Research and Assessment (ORA) and Gift of Life Michigan, recently collaborated in the development of an automated reporting system for organ and tissue referrals. Washtenaw County uses the MDILog® web-based software service (developed by ORA) for its case management, both hospital (University of Michigan) and non-hospital medical examiner cases are entered in the system as they occur. Gift of Life traditionally contacts the Washtenaw medical examiner's office each morning to inquire about recent cases and their potential for referral. The goal of the system was to reduce medical examiner staff time in fulfilling the mandated reporting requirement and increase the number of referrals to Gift of Life Michigan and the number of cases that resulted in actual donated organs and tissue. In addition, the resulting data transfer protocol (schema) could be shared with anyone wishing to replicate the data exchange process with their own case management system and the state/local organ procurement organization they are required to report cases to.

The concept is simple: using the investigative data set, the Organ Procurement Organization (OPO) (in this study Michigan Gift of Life) identifies which case criteria are essential to initiate a case for procurement. If the investigators have the correct hardware (laptop/tablet with cellular connection), they are able to connect to the case management system and start processing the case immediately on arrival at the scene. Once (and if) OPO case data thresholds are met and saved, the data flows seamlessly (without humans in the loop) to the Gift of Life computer center where case screeners review case details (only essential data is viewable to OPO staff) and provide feedback to the investigator (hold or release), allowing medical examiner staff to coordinate timely release of the body to funeral directors or tissue procurement agencies. If the suspected cause and manner of death require additional forensic investigation and autopsy, the investigator simply checks a box informing the OPO of a medical examiner "hold" on the referred case.

In the first six months since going "live," the automated data exporting system has processed 56 totally automated referrals resulting in 8 additional tissue donors. Gift of Life concluded that: implementation of the program required a review of internal case processing procedures and that it is likely that two of the cases resulting in donation would have otherwise been lost due to unnecessary time delays. Automated data exchanging avoids duplication of effort, human error, and saves time and tissue.

Tissue Donors, Automated Reporting, Organ Procurment

G112 A Retrospective Study of Non-Criminal Autopsy Cases in the Central Part of China From 2001–2010

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After attending this presentation, attendees will gain knowledge about the medicolegal death investigation system in China and common causes of sudden unexpected death, accidental death, and suicides in central part of China.

This presentation will impact the forensic science community by showing the importance of forensic autopsy in the medicolegal death investigation. The understanding of deaths due to allergic reaction to antibiotics caused by medical malpractice could lead to the prevention of such deaths.

Medicolegal death investigations in China are conducted by forensic medical experts, mainly within five relatively independent agencies: (1) the police departments; (2) the prosecutors' offices; (3) forensic medicine/science institutes in medical colleges and universities; (4) Institute of Forensic Science in the Ministry of Justice; and, (5) the government or private forensic societies. Approximately 300 forensic medical experts are distributed in 33 of 150 medical colleges, four universities of political science and law, and nine colleges of police or criminal police. The forensic medical experts/forensic pathologists in the medical colleges and universities are primarily responsible for the medicolegal examination of sudden unexpected deaths, accidental deaths, and suicidal deaths.

Wuhan is the capital city of Hubei province in central China with a population of 9,785,392. Forensic pathologists at Tongji Forensic Medicine Center of Huazhong University of Science and Technology (TFMC) are primarily responsible for the forensic examination of the sudden unexpected deaths, accidental deaths, and suicide cases in Wuhan. A retrospective study of forensic autopsy cases was conducted at the TFMC from 2001 to 2010. A total of 854 autopsy cases were performed by the forensic pathologists at TFMC in Wuhan city during the ten-year period. Of these deaths, 799 cases were determined to be non-criminal deaths. The age of individuals ranged from 0 to 82 years with the majority of subjects in the ages of 21 to 60 years (560 cases, 70.1%) and the mean age of 33.79±19.17 years. As regards to gender, there was a male preponderance (male: female = 1.87:1). More than 60% of deaths were due to natural diseases (488 cases), 213 cases (26.7%) were from accidents, and 28 (3.5%) were suicide. Of the 799 cases, the cause and manner of death could not be determined in 70 cases (8.8%) after thorough death investigation and autopsy examination. Of the natural deaths, 209 subjects (26.2%) died of atherosclerotic cardiovascular diseases and 31 subjects (3.9%) died of intracranial hemorrhage. There were 113 cases (14.14%) involveding infant deaths. Asphyxia due to amniotic fluid aspiration was the most common cause of death in newborn babies. Interstitial pneumonia was the most common cause of death in infancy. Sudden Infant Death Syndrome (SIDS) was a very uncommon diagnosed cause of death in infants. The study also showed that massive bleeding after delivery was the major cause of

death in the perinatal women. Of the 799 cases, 213 cases were accidental deaths. Of the 213 accidental deaths, 42 deaths were due to motor vehicle accident, 22 deaths were due to adverse drug reaction caused by medical malpractice, most of which resulted from allergic reaction to antibiotics. There were five cases from anesthesia accidents.

In summary, this report focuses on the characteristics of the non-criminal deaths occurring in central China. Presented data indicate that atherosclerotic cardiovascular disease is the number one cause of sudden unexpected natural deaths in Wuhan, followed by intracranial hemorrhages. Interstitial pneumonia was the leading cause of death in infants. Deaths due to allergic reaction to antibiotics caused by medical negligence remain a serious health problem in China.

Forensic Autopsy, Cause of Death, Drug Reaction

G113 Pathologic Study of 125 Autopsied Cases Where Subjects Died in Custody

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After attending this presentation, attendees will understand relative information about death in custody in China and the fact that most of these deaths are natural without homicide and illegal factors.

This presentation will impact the forensice science community by discussing impressions of Chinese prison law enforcement.

In recent years, the people who have died in custody or during the law enforcement practices have extensively attracted public concern. The possibility of violation of human power or illegal practices by police officers and other law enforcement officers have been frequently questioned. For this reason, a retrospective study was performed on the 125 cases that died in different sites such as prison, custody, detention centers, drug addiction treatment places, interrogation rooms, detention rooms, as well as during arrest and other enforcement processes of the public security bureau, prosecutors, courts, and other units. The 125 cases were selected from the Department of Forensic Pathology, Tongji Medical College, Huazhong University of Science and Technology from 1999 to 2009. For all of the cases, a comprehensive autopsy and histo-pathological examination were performed, some of them with laboratory tests such as toxicological analysis or biochemical examination of blood. Results: (1) the cases who died in the above-mentioned sites accounted for 4.42% of cases in 11 years, and showed a declining tendency in the past four years; (2) gender and age: men (123 cases, 98.40%) obviously more than women (2 cases, 1.60%), aged between 15 and 77 years old, with young and adult male from 20 to 49 years old occupied 103 cases (82.4%), the two female cases were 23 and 42-years-old; (3) occupation: 72 cases (57.6%) were farmers; 30 cases unemployed; worker, cadre, student, and self-employed, were four, three, two, and one cases, respectively; and the remaining 13 cases were occupation unknown; (4) Duration in detention: from zero hours to five years and seven months, mostly less than one month (68 cases, 54.4%), 99 cases in six months (79.2%); (5) Interval time between death and autopsy: five hours to 209 days, of which 60 cases were within 48 hours (48%); (6) the place of death: hospital, prisons, detention centers, interrogation rooms, drug addiction treatment places, arrest and elsewhere were 79, 14, 12, 8, 3, 2, and 7 cases respectively, with the majority (63.2%) dying in hospital; (7) Cause of death: among 118 known cases, 86 died of diseases; 16 cases, mechanical asphyxia; 13 cases, mechanical injury; three cases, poisoning; (8) manner of death: 86 cases were natural deaths; 25 suicide; seven accidental death; seven unknown; and no homicide cases; and, (9) classification of diseases: in 86 cases death of of disease, 41 were from cardiovascular diseases; 17 from respiratory system diseases; nine, central nervous system diseases; eight, digestive systems diseases, and 11 cases, other diseases. This data is consistant with the facts that most criminal offenders are young and adult men, the majority of deaths are natural except for a small number of suicides and accidental deaths, and their were no homicidal cases. It is suggested that all police and law enforcement units should further strengthen education, management, and disease prevention in order to prevent suicide, accidents, and illnesses.

Forensic Pathology, Regulatory Sites, Analysis of Death

G114 When Is a Murder Not a Murder? When It's a Non-Suspicious Death!

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After attending this presentation, attendees will understand the problems faced by forensic pathologists in the United Kingdom (U.K.) resulting from the distinction between "suspicious" and "non-suspicious" deaths, and the steps that can be taken to reduce the risks of homicides "slipping through the net."

This presentation will impact the forensic science community by highlighting the importance of educating all professionals involved in the medicolegal investigation of death regarding the importance of suspicion that a death may be related to criminal activity at the earliest stage of an investigation.

Unlike most jurisdictions, the U.K. divides autopsy practice into "suspicious" and "non-suspicious" deaths. As a result of this, the vast majority of medicolegal autopsies undertaken in England and Wales are performed by pathologists with limited, or in many cases no experience of the appropriate approach to potentially homicidal deaths and indeed many have never even observed a "suspicious death" autopsy. Furthermore, there is a likelihood that if a death is referred for autopsy as non-suspicious, then the pathologist is falsely reassured that there is "nothing to worry about."

Of course, some potential homicides are not identified as a result of the initial investigations, and are referred for a "routine coroner's autopsy." At the very least, this can lead to compromise of forensic evidence as the scene is not preserved and the body is not transported in a manner that preserves trace evidence. The forensic autopsy may be more challenging if an autopsy has already been commenced or even completed before the death is recognized as one that may require a more detailed examination than usual.

To demonstrate the potential pitfalls of this approach to autopsy and the difficulties it causes for the forensic pathologist and the wider aspects of a criminal investigation, three relevant cases will be discussed.

In the first case, a man with a history of alcohol abuse was found dead in a moderately advanced state of decomposition with a significant postmortem interval. The death was referred for a routine autopsy and, as there were no close relatives, after the examination the body was frozen awaiting the funeral. However, as the estate was being settled, it came to light that money had been taken from the deceased's account and benefits claimed for a considerable period after he was believed to have died. A suspect for this fraud was developed but a forensic autopsy was requested to exclude "foul play" in the death as well as after it.

The second case involves a body recovered from a river. The case was investigated by a junior police officer and deemed to be non-suspicious despite external injuries that were considered to be "unexplained but not suspicious." External examination by a general pathologist apparently revealed no marks or injuries to cause concern, but on commencing the internal examination the pathologist was faced with a situation he had not considered and a forensic examination was only requested a week after discovery of the body.

The final case revolves around an unexpected death in a nursing home. On commencement of the initial autopsy, the pathologist became very concerned and asked for assistance from a forensic pathologist who was able to confirm that the death was almost certainly the result of an assault and triggered a criminal investigation.

Finally, this presentation will address the ways in which these problems can be alleviated and how the East Midlands Forensic Pathology Unit (EMFPU) in Leicester is attempting to minimize the issues by education of all the relevant professionals, from police officers to trainee general pathologists. This approach should benefit the interests of justice and prevent pathologists who are inexperienced in forensic pathology from being exposed to an adversarial justice system for which their training leaves them unprepared.

Homicide, Suspicious Death, Education

G115 The Temporal Dynamics of Autopsies and Their Impact on System Efficiency: Do Autopsies Really Come in Threes?

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After attending this presentation, attendees will understand the temporal fluctuations in the rate of autopsies, the accordion effect as it applies to pipeline processes, and how these impact efficiencies in the practice of pathology, both forensic and hospital-based.

This presentation will impact the forensic science community by: (1) providing insight into the forces internal and external to a laboratory which affect its processes and systems; and, (2) providing a framework for developing strategies to mitigate those effects and increase departmental productivity.

Barring mass disasters or epidemics, patients for autopsy generally arrive in both forensic and hospital-based pathology departments randomly over time. Within this random framework, however, there is a definite propensity for temporal clustering of autopsies, lending credence to informal observations by pathology residents that "autopsies seem to come in threes."

This temporal clustering provides a practical example of the "accordion effect." The accordion effect is well known in traffic science, foot marching, and bicycle racing, and it can affect processes in a pipeline in general. Similar to fluid dynamics in physics, the accordion effect creates fluctuations in sequential or pipeline work processes, creating disruptions in the flow of processes following it. Using traffic as an example, say a driver changes lanes suddenly. This sudden interruption in flow, followed by many cars applying brakes, creates a so-called "stop wave," which can travel backward to delay all the cars that follow.

Autopsy pathology is generally conducted as a series of processes in a pipeline fashion, making it susceptible to the accordion effect. A typical autopsy pipeline looks like this: the autopsy is performed, tissues are submitted to a single department for histology, microscopic slides are returned to the same pathologist, who provides information to clerical personnel, who return the work to the pathologist for final revision, culminating in the issuance of a completed report. A slowdown anywhere in the system has a direct "downstream" effect on every process that follows it, similar to the backward traveling wave of delay on the highway.

When the federal government mandated prospective payment systems for Medicare reimbursement in 1983, medicine began to experience a cultural shift. Facing serious fiscal cutbacks, hospitals needed to find ways operate more efficiently to survive. For the first time, health care delivery systems began to assess efficiency and quality in medical care using tools traditionally used in industry, such as time-motion studies and total quality management techniques. Although not the recipients of Medicare funding, most forensic pathology departments are government-based and operate with limited fiscal resources and staffing. Both hospitals and forensic laboratories face substantial challenges to system efficiency, cost-effectiveness, and quality.

A historical prospective study of autopsy frequency in a university hospital pathology department between the years 2001 – 2010 was conducted. The number of autopsies varied from 63 to 107 per year. The average number of autopsies performed each month within a given year ranged from six to eight, with a range of zero to nineteen per month.

An empirical analysis of autopsy clustering, factors potentially creating ebbs and flows in the autopsy process, and how the information may help guide institutional strategies for process improvement will be presented.

Autopsies, Accordion Effect, Efficiency

G116 Till Death Do We Dye?

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The goal of this presentation is to determine a meaningful consensus on reliability and validity of this adjunct, in order to better evaluate current practice.

This presentation will impact the forensic science community by showing: (1) improved outcome in service for victims of gender-based violence, a recognized public health issue (CDC, 2009); (2) improved examination methodology, greater diagnostic acumen, more accurate documentation, and better communication between homicide investigation teams and forensic science and public health communities; and, (3) increased transparency, efficiency, communication, and reduced health disparities for marginalized groups of victims, e.g., prostitutes, victims of human trafficking, and undocumented immigrant women.

The interpretation of genital findings in the deceased is both timely and pivotal. Gender-based violence is both a domestic and global issue. The focus of the present discussion is to discuss the use of 1% aqueous toluidine blue dye as an adjunct to the evaluation of the sexual assault victim. This discussion is based on a review of the literature of toluidine blue dye in *living* sexual assault victims *and* results of dye application as part of an exploratory study on fatal sexual violence against women, using a cross-sectional, prospective design and a convenience sample of 74 females: 46 from the Body Donation Program at University of California, Davis Medical school; 18 coroner's cases; and ten homicide cases, ranging in age from 24 months to 75 years.

Toluidine blue dye, a nuclear stain, has been widely recommended, adopted as a practice standard, and often recommended in local, regional, national, and international guidelines for sexual assault victims, including the Department of Justice (DOJ), Agency for Healthcare Research & Quality (AHRQ), American College of Emergency Physicians (ACEP), New England Journal of Medicine (NEJM), World Health Organization (WHO), and the International Association of Forensic Nurses (IAFN).

With increasing frequency, new studies on genital injury in *living s*exual assault victims incorporate dye in their methodology. Each new study builds upon the assumption that both reliability and validity of the dye have been recognized. Most of these authors cite original, well-regarded studies, including Richart,[1] Collins,[2] and Lauber & Souma,[3] as foundational cornerstones. As the yield of studies expands, it may be useful to ask questions germane to accurate interpretation: Have the reliability and validity of toluidine blue dye for application on various sites of the anogenital anatomy been sufficiently studied to warrant the current recommendations and practice guidelines for examination of sexual assault victims? In this *Fatal Sexual Violence Against Women* study, most of the 46 cases from the Body Donation Program were examined with both colposcopy at 7.5X and 15X magnification and a 35mm Single Lens Reflex (SLR) digital camera.

Dye was applied to 30/46 subjects, with 100% false-positive dye uptake. Richart[1] and Collins[2] noted 23 benign conditions that caused false-positive results in their samples. Current studies often question the admissibility of evidence, e.g., reliability of colposcopy, citing *Daubert and Federal Rules of Evidence,*[4] *versus Kelly-Frye*.[5] Colposcopy is a modality of binocular microscopy, with the capacity for magnified digital, film, and/or video photographs.

Are colposcopes photographs inherently different types of physical evidence? If reliability and validity is well established for both film and digital imaging, does this transfer to images viewed through colposcopic optics and captured by cameras? Toluidine blue dye is far less costly than colposcopy. As a quality indicator, what level of influence does this have on outcome? Another consideration is an examiner's expertise. Detailed information on sexual anatomy, even in living victims, is still a young field, especially when contrasted with other forensic specialties. Less experienced examiners may benefit from methods that include magnified photographs, whether colposcopy, or other digital systems.

- In a review of the original, core, studies on toluidine blue, the following issues were found to be germane: Dye requires meticulous technique; decolorization is more important than application.
- Interpretation of findings is subjective. Richart,[1] Collins,[2] and Lauber & Souma,[3] all used *qualitative* terms, such as *deep royal blue*, (positive), or *diffuse and/or patchy uptake* (non-specific), to describe patterns of dye uptake; these are *descriptive*, but also non-specific and subjective.
- Original study findings were *confirmed by biopsy*. The majority of subsequent studies on genital injury in adult/adolescent female subjects did not incorporate *follow-up* examinations, even when acute trauma was present.

Follow-up would effectively use the victim or study subjects as their own control.

Much has been learned about sexual anatomy and injury in female sexual assault victims. Concomitantly, the capacity to compare and contrast *normal* with various other cohorts increased. Toluidine blue dye was included in the initial iteration of the *sequential methodology for postmortem genital examinations*.[6] After further study, recommendations for dye application were removed from later iterations and publication.[7] A review and consensus by subject matter experts could evaluate the pivotal issues of reliability and validity. Such a panel could also facilitate the comparison and contrast of "cornerstone" results to those of recent studies. Individual anatomic sites could be reviewed for histological similarities/differences, to help determine appropriateness, applicability, and generalizability.

The ultimate goal of this endeavor is to improve the understanding of what is *normal*, *and what is not*, for the anogenital anatomy during the postmortem interval. The astute examiner must perform these examinations with optimal expertise and chronicle vital data. In this manner, the capacity to understand fatal sexual violence against women will continue to grow.

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Gender-Based Violence, Toluidine Blue Dye, Colposcopy

G117 An Unusual Mechanism of Bladder Fluid Absorption With Fatal Fluid Overload in "Predisposed" Patient

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After this presentation, attendees will be aware of the possible clinical and legal implications following urothelial damage and fluid absorption

This presentation will impact the forensic science community by showing an unknown mechanism of fluid absorption with remarkable risk of complications due to fluid overload in "predisposed" patients.

An 85-year-old man had been undergoing hemodialysis treatments three times a week for the past three years. He was a water-controlled patient, not self feeding. One day he presented hematuria and the doctors decided to perform a continuous bladder irrigation. During the first day of irrigation, the patient did not have any problems and underwent his hemodialysis treatment as usual. Twenty-four to thirty-six hours after hemodialysis, while still undergoing irrigation, the patient gained 7.4kg. His wife said that the fluid introduced by bladder irrigation was not expelled, so the only explanation of this weight gain was the bladder irrigation procedure. Blood test results were as follows: Na^{+} 130 ↓ *(vn 130-148 mEq, intermedial data T1: 111, T2: 116, T3: 126),* $HCO3^{-}$ 19,8 ↓ *(vn 23-25 mEq, intermedial data T1: 16,2, T2: 17,8, T3: 22,5);* Ph 7.36 ↓ *(vn 7.35-7.45, intermedial data T1: 7.23, T2: 7.34, T3: 7.38),* PCO_2 98 *(VN 38-42 mmHg).* These data show a mixed acidosis due to hypervolemia, responsible for the reduction of sodium and HCO3- and for the development of pulmonary edema which led to an alteration of the ventilation and a CO_2 increase. Unfortunately, the clinical situation deteriorated rapidly and the patient died. The autopsy revealed hypertensive heart disease with increase in weight (660g); the lungs weighed 790g (right) and 590g (left) and were congested and edematous. There was a presence of coagulated blood and heavily congested mucosa in the bladder.

Histological examination of the bladder revealed the absence of the urothelium, with tunica propria affected by a series of alterations likely to be explained in the context of an erosive inflammation (necrotizing). Correct functionality of urothelium is fundamental because it is impermeable to fluid, thus being an effective barrier against the passage of osmotic fluid from blood into urine, and has the capacity to adapt to the bladder volume. Damage or loss of the epithelial surface by chemical, mechanical, or other mechanisms implies a reduction or total loss of this important insulation function provided by an intact urothelium.

A condition of abnormal resorption at the vesico-urethral level can occur during TURP: transurethral incision may determine vascular lesions, creating conditions for the absorption into the circulation of the liquid used for washing during surgery. Resorption can determine the onset of actual pathological syndrome, even a serious one, defined precisely as TURP syndrome.

In conclusion, the loss of the urothelium led to an alteration in the permeability of the bladder and to an abnormal resorption of fluid resulting in hypervolemia. This was the cause of mixed acidosis and massive pulmonary edema which led to *exitus*.

Bladder Absorption, Hypervolemia, Acidosis

G118 Fatal "Shaken Adult" Syndrome? A Case Report of an Elderly Patient

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After attending this presentation, attendees will be aware that shaking an elderly person's head might be fatal, causing brain damage in a way similar to shaken infants, and that a subdural hematoma of unknown origin in the elderly should raise the possibility of abuse.

This presentation will impact the forensic science community by raising awareness of the possibility of the existence of a new forensic pathology entity which might be called "Shaken Granny Syndrome." This will participate in a more global goal which is to increase the awareness of prevalence of elder abuse, clearly underestimated, in the forensic community.

An 81-year-old man suddenly lost consciousness and collapsed after a 45 minute quarrel with his wife and her lover, who reported to the police that they shook him back and forth several times, by grabbing his clothing, his shoulders and arms, without any blow to the head. Despite swift rescue action, he was pronounced dead at the scene.

He had been prescribed anticoagulants for a pulmonary embolism for years and an MRI, taken six months before death for behavior and memory disorders, showed extensive grey matter atrophy.

The autopsy performed the next day showed only recent bruising of the anterior chest and upper limbs. There was no hematoma of the inner aspect of the scalp, no fracture of the skull. A "fresh" subdural hematoma of eighty milliliters, non-adherent to the dura matter and located in the left hemisphere, was found without brain contusion at the macroscopic exam. Besides a non-occlusive coronary atherosclerosis, the other internal organs were unremarkable. Toxicology was negative (presence of treatments at therapeutic levels).

Basic histological examination showed a neutrophilic infiltrate of the bruising, thus considered as peri-mortem. The subdural hematoma was mainly composed of non-lysed red blood cells, which was consistent with an acute and recent onset. A minor subarachnoid hemorrhage and some cortical "flame-shaped" hemorrhages were found, suggesting that the subdural bleeding was traumatic. There was, however, no focal contusion of the brain and, therefore, no argument in favor of a direct trauma of the head. Histological examination of the other organs was noncontributory.

Selected brain sections were prepared for complementary immunohistochemical examination using β Amyloid Precursor Protein (β-APP) antibody, showing Diffuse Axonal Injury (DAI) scattered in different brain areas. Eyeball collection not being (so far) a routine procedure in adults, was impossible to check for retinal hemorrhages (the interest of which will be discussed during the presentation, focusing on specificity concerns). Cause of death was classified as "traumatic brain lesions and hemorrhage," and manner of death as "homicide."

In this case, trauma was considered the result of acceleration-deceleration forces due to brutal movements of the head secondary to violent shaking.

The Shaken Baby Syndrome is well known, but a literature review showed that the "Shaken Adult Syndrome" is still to be described. Only one case of a young adult who died from brain damage after his head was violently shaken during police custody was found during this study.[1] In the absence of the research of DAI, this case was questioned.[2]

Because of anticoagulant prescriptions, weakness of cervical muscles and some extra space between the brain and the cranial bone due to cerebral atrophy (with tensioning of the bridging veins within the subdural space), this case shows some similarities with the conditions found in the physiopathology of the "Shaken Baby Syndrome".

These conditions being often met in the oldest patients, the concept of "Shaken Elderly or Granny (rather than adult) Syndrome" might be relevant. This is of special interest when one considers that in some series, 10% of subdural hematomas of the elderly are "spontaneous", i.e., of unknown origin, the hypothesis of physical abuse being then exceptionally (or never) raised in the publications.[3]

Further studies are needed to better characterize the prevalence of subdural hematoma in elder abuse and the percentage of cases related to "shaking."

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Subdural Hematoma, Elder Abuse, Shaking

G119 Autonomic Nervous System and Sudden Death

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After attending this presentation, attendees will improve their understanding about "unexplained" sudden deaths with negative autopsy findings caused by the activation of the utonomic nervous system, as well as cardiac deaths following injuries to other organs and related to the effects of the autonomic nervous system.

This presentation will impact the forensic science community by providing detailed knowledge about the most common mechanisms of death following activation of the autonomic nervous system.

Although the cardiovascular system is often considered a unique physiological entity, the evidence of a link between sudden cardiac death and autonomic nervous system is compelling. It is in fact well recognized that physical and emotional stress (such as anxiety or anger) may lead to sudden death as a result of arrhythmias, myocardial ischaemia, and infarction. Experimental evidence from human and animal models proves that stimulation of certain brain areas (mainly the insular cortex, the infralimbic cortex, and the amygdala) or sympathetic efferent fibers to the heart can produce fatal abnormaities of the heart rhythm. Psychiatric diseases are also known to cause sudden cardiac death. There is integration between the limbic system and the cortical autonomic "control sites." Circuitry between these areas might result in abnormal electrical stimulation to the SA node resulting in sudden death.

This catastrophic event is known to be responsible for more than 300,000 sudden cardiac deaths every year in the United States. The majority of victims are thought to have suffered ventricular tachycardia or ventricular fibrillation. Several clinical and experimental studies suggest that heart rate variability and baroreflex sensitivity are the most reliable predicting factors for possible future cardiac events.

Central autonomic dysfunctions causing cardiovascular complications such as ECG changes, cardiac arrhythmias, ischaemic damage to the myocardial muscle, and disturbances of blood pressure regulation might often occur as a result of brain injuries or acute cerebrovascular disease. These often life-threatening complications are thought to be due to increased sympathetic tone with subsequent elevation of circulating catecholamines. Several experimental and human studies have suggested that the most

arrhythmogenic areas, if injured, are the prefrontal areas or the insula. These brain structures might also give rise to further cardiac complications involving heart rate variability and possible heart failure. Although head trauma-induced heart rate variability has been recently studied with modern techniques, cardiac complications following brain disease are now being redefined.

The ECG changes following brain damage or ischaemia can often mimic myocardial ischaemia. The most commonly described abnormalities are regarded to be ST segment depression, flat or inverted T waves, prolonged QT intervals, and U waves. These phenomena have been proved to be due to autonomic nervous system reaction to the brain disease, and are not related to any kind of heart or coronary artery disease. In comparison with cardiac related ECG changes, these seem to appear later, with a peak about two days later from the cerebrovascular event (or trauma) and revertion within two weeks. It is interesting that, although in the majority of cases the ECG changes do not reflect real myocardial damage, sometimes macroscopic and microscopic changes to the myocardium have been observed at autopsy without coronary artery disease. These changes were observed to be myocardial necrosis with histiocytic infiltration, subendocardial hemorrhage, and myofibrillar degeneration.

Sudden Death, ANS, Forensic Autopsy

G120 Plaque Morphology in Subjects With Coronary Disease Who Suddenly Died

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After attending this presentation, attendees will be able to better understand the main pathological features of atherosclerotic plaque and pathogenesis of artery thrombosis in coronary sudden death.

This presentation will impact the forensic science community by increasing awareness that Sudden Coronary Death (SCD) depends frequently on active atherosclerotic lesions.

Most of Acute Coronary Syndromes (ACS) are precipitated by luminal thrombi, which arise from three different plaque morphologies: rupture, erosion and calcified nodules. Of these, plaque rupture is the most frequent, accounting for 60 – 75% of cases. The purpose of the present study was to determine the frequency of active and inactive coronary lesions and Myocardial Infarction (MI) in people with SCD.

SCD is defined as a sudden unexpected death within one hour of onset of acute symptoms from a stable medical condition or the death of a person who had been seen in stable condition less than 24 hours antemortem. No other potentially lethal cardiac or noncardiac cause of death could be present, including toxicology screening. The presence of acute thrombosis (collections of platelets, fibrin, and trapped erythrocytes and white blood cells) and disrupted coronary plaques (disruption of the luminal fibrous cap with fissure or rupture into a lipid core) was noted. An active coronary lesion was defined by the presence of a disrupted coronary plaque, luminal thrombus, or both (luminal thrombus in the area of a ruptured plaque). An inactive lesion had a luminal stenosis ≥75%, but lacked both plaque disruption and thrombus. Organized thrombi consisted of granulation tissue and recannalized channels within the arterial lumen with or without fibrin. Healed MI was identified by focal macroscopic replacement of the myocardium by scarring, with histological confirmation. Acute MI was diagnosed by the presence of coagulation necrosis with or without an associated inflammatory infiltrate.

The distribution of coronary lesions for the entire group of hearts (166 cases) was: acute thrombosis in 87 (53%), disrupted coronary plaque (with or without acute thrombosis) in 54 (32%), and organized thrombus in 25 (15%). There were also active coronary lesions in 49 cases (30%). The association of thrombosis and disrupted coronary plaque was as follows: acute thrombosis with plaque disruption in 49 cases (30%) and acute thrombosis without plaque disruption in 38 (23%). Disrupted plaque without acute thrombosis was identified in 5 cases (3%) among the 72 coronary lesions with ≥75% luminal stenosis. Plaque hemorrhage of any size was present in 96 cases (58%); of these, plaque hemorrhage occupying ≥25% of total plaque area was identified in 89 (54%). Thus, SCD could be attributed to active coronary lesions (thrombus, disrupted plaque, or both) in 141 cases (85%). There were 22 cases (13%) without an active coronary lesion which had an acute (two cases) or healed (20 cases) MI. In the 34 cases without an acute or a healed MI, 17 (50%) had only inactive coronary artery lesions such as severe atherosclerosis without acute coronary thrombosis or plaque disruption. The examination of the myocardium revealed acute MI only in 16 hearts (10%), both acute and healed MI in 18 (11%), healed MI in 68 (41%), and the absence of MI in 63 (38%). In hearts with acute MI, the infarct was identified macroscopically in 9 of 27 cases. Of these 27, there was a transmural infarct in 20 cases and subendocardial in seven cases. Platelet-fibrin emboli in small intramyocardial coronary arteries were found in 13 cases (8%) and only in cases with an acute coronary thrombosis.

Acute changes in coronary plaque morphology (thrombus, plaque disruption, or both) were found in 85% of SCD cases. In hearts with myocardial scars and no acute infarction, active coronary lesions were identified in 31 (46%) cases. Neither myocardial infarction (acute or healed) nor active coronary lesion was present in 38 (53%) cases.

Sudden Death, Atheroscler Plaque, Myocardial Infarction

G121 Aneurysms of the Coronary Arteries and Sudden Death: Three Case Reports

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After attending this presentation, attendees will be able to better understand the main pathological features and pathogenesis of Coronary Artery Aneurysms (CAAs) in atherosclerotic—one case, and Kawasaki Disease (KD)—2 cases.

This presentation will impact the forensic science community by increasing the awareness that CAAs bear a significant risk of sudden cardiac death and that their development depends on transmural vascular inflammation.

CAAs are rare with an incidence of 0.02% to 0.04% in the general population. They are most commonly associated with the male gender and hyperlipidaemia. The proximal right coronary artery seems to be predominantly altered. About one-fourth of these patients have multiple coronary aneurysms. CAAs most commonly develop secondary to atherosclerotic coronary artery disease. Some CAAs are described as congenital and are linked to KD. Other circumstances are rather exceptional (trauma and dissection, infectious, scleroderma, connective tissue disorders such Marfan or Ehlers-Danlos syndromes and lupus, neurofibromatosis, periartheritis nodosa, and Takayasu's arteritis). Intraluminal (parietal or occlusive) acute thrombosis is found in 75% of the aneurysms with possible development of myocardial ischaemia and Sudden Death (SD). SD may also occur subsequently after rupture of CAAs and cardiac tamponade.

The first case regards a 3-month-old Italian male baby who, three days after the compulsory vaccination and antipyretic administration to calm the feverish condition, gradually showed eyelid conjunctival and lip erythema, diffuse cutaneous exanthema, and pharingo-tonsillitis associated to staring episodes. At admission, his temperature was 39°C and the hemato-chemical data were: erythrocytes $3.51x10^6$/uL; leukocytes $9.92x10^3$/uL; platelets $626x10^3$/uL; alcaline phosphatase 334 IU/l, PCR 7.85mg/dl; ALT 49IU/l. An echocardiogram showed a diffused hyperechogenic right coronary, dilated in the proximal tract with a 4mm aneurismal margin; hyperechogenic left coronary, dilated at the level of the common trunk,

with an aneurysmatic aspect at the level of the first tract of the anterior interventricular septum; moderate pericardial effusion; well preserved myocardial contractility. Death suddenly occurred nine days after admission. The autopsy revealed that death was due to cardiac tamponade subsequent to aneurysm rupture of the anterior descending coronary, the wall of which showed active transmural lymphomonocytic inflammation. The second case regards a 2-month-old male Indian infant. The infant showed signs of rhinitis and coughing associated with conjunctival hyperemia and allergic exanthema on the chest and arms. Laboratory analyses showed: leukocytosis (15.370/mm^3), elevated sedimentation rate; positive C-reactive protein, and thrombocytosis (476.000/mm^3). Klebsiella pneumoniae was isolated from the urine and an antibiotic therapy was started. Coughing, rhinitis, exanthema, and conjunctivitis progressively decreased until they disappeared, while laboratory tests showed an increase in phlogosis indices. Death came suddenly and unexpectedly on the seventh day of recovery. The autopsy revealed that death was due to cardiac tamponade subsequent to aneurysm rupture of the anterior descending coronary, the wall of which showed active transmural lymphomonocytic inflammation.

The third case concerns a 66-year-old man with a history of ischaemic heart disease and stroke cerebri, who entered the emergency area in a stupor state. The patient became comatose (Glasgow Coma Scale: 5) and the brain CT scan demonstrated a hyperdense right middle cerebral artery with a flattening of the cortical sulci along with signs of a suffering chronic hypoxia and after effects of an extensive malacic bilateral frontal area. The death occurred four hours after recovery. The autopsy showed a diffuse fibro-atheromasic thickening of the walls of the subepicardial coronary arteries which appeared focally aneurysmatic along with occlusive endoluminal thrombosis and chronic transmural flogosis.

The pathomechanism of CAAs remains a controversial topic of discussion. Weakened media of the coronary wall with a diminution of its elastic elements in areas of severe atherosclerotic plaques, and intraluminal pressure against the defective vessel wall, allow the vessel to dilate progressively. Not only does the chronic transmural inflammation destroy the media which represents the pathomechanism of CAAs in KD, but also makes an analogous possible pathway in atherosclerotic aneurysms. Our cases show that CAAs are an independent predictor of mortality because their development depends on the possible transmural extent of intimal (in atherosclerotic disease) and adventitial in KD inflammation. MRI represents the best diagnostic tool for identifying this rare pathological evolution.

Coronary Aneurysms, Atheroscler Disease, Kawasaki Disease

G122 Sudden Cardiac Death Due to Acute Coronary Artery Dissection Following Exercise-Related Blunt Chest Trauma: A Case Report

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After attending this presentation, attendees will learn about an unusual case of unexpected cardiac death during sports activities due to an acute coronary artery dissection following blunt chest trauma.

This presentation will impact the forensic science community by increasing awareness of sudden cardiac death following a blunt chest trauma occurring during sports activities.

The case is presented of a healthy 43-year-old man who suddenly collapsed ten minutes after the beginning of his sporting activity, kung fu. Despite resuscitation efforts by paramedics, the patient never regained a cardiac rhythm and expired. Review of the decedent's hospital records revealed that he had no specific past medical history. A forensic autopsy was performed 48 hours after death. At the external examination, the body showed embalming. There were also some contusions on the upper limbs, and one contusion on the left lateral chest area. Area of hemorrhage were found next to the left lateral fourth, fifth, and sixth intercostal spaces and anterior to the left second. The aortic arch and the descending aorta was normal and without coarctation. The heart weighed 410g which was within the normal reference range, and it had unremarkable myocardium and cardiac valves. The coronary arteries were present in a normal distribution with a right dominant pattern. An area of hemorrhage next to the proximal segment of the left anterior descending artery was observed. The other coronary arteries were without abnormalities. The lungs were edematous and their cut surfaces were congested. The gross examination of the left anterior descending artery showed a thick, recent adventitial hemorrhage surrounding the proximal segment with luminal obstruction by hemorrhage. Histological examination of the left anterior descending coronary artery revealed an acute dissection between the inner four-fifths and outer one-fifth of the media with collapse of the lumen due to blood in the false channel. Histologic examination revealed no evidence of inflammation, medial degeneration, or vasculitis. The arterial wall not involved by the dissection was otherwise unremarkable. An elastic stain showed essentially normal elastic fibers. In addition, the postmortem toxicological screening was negative. The death was attributed to an acute dissection of the left anterior descending artery resulting in an acute myocardial ischemia.

The heart's position between the sternum and vertebral column makes it vulnerable to injury from blunt chest trauma. Cardiac injuries after blunt chest trauma are various, including myocardial contusion or hemorrhage, arrhythmia, cardiac rupture, valvular injury, and acute myocardial infarction. Road traffic accidents are mainly responsible for blunt chest trauma and heart injury. Coronary artery dissection following blunt chest trauma is rare and is even less common in the setting of a contact sport such as kung fu. Coronary artery dissection following blunt chest trauma typically involves the left anterior descending artery; the probable explanation is its vulnerable anatomic position on the anterior part of the heart. The second most commonly affected artery is the right coronary artery. Although rare, left circumflex coronary artery involvement has also been reported. Shearing forces during the traumatic episode probably cause intimal tears of the most vulnerable part of the left anterior descending artery, which subsequently initiates the process of thrombus formation. Cases of coronary artery dissection have been described after practicing rugby, soccer, basketball and water-skiing. It is assumed that this is the first report of acute coronary artery dissection leading to sudden death following the practice of martial arts. The martial arts are considered relatively safe compared to many other sports, including football, basketball, and wrestling, and most martial arts injuries reported in the literature are minor. Indeed, the most common types of martial arts injury are sprains, strains, and contusions, and the less common injuries include fractures, dislocations, and dental injuries.

In conclusion, the forensic pathologist may be aware of the possibility of sudden cardiac death in a context of martial arts practice, and that martial arts are mistakenly considered safer than most.

Coronary Dissection, Sports, Blunt Chest Trauma

G123 Evaluation of Prevalent Ischemic Damage at the Right Ventricle Compared to the Left One: Improvement of a Diagnostic Tool for the Diagnosis of Fatal Pulmonary Fat Embolism?

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After attending this presentation, attendees will improve their knowledge on the physiopathology and pathology of cardiac damage after pulmonary Fat Embolism (FE). They will learn how immunohistochemistry can be helpful in the diagnosis of fresh ischemic cardiac changes and they will learn how to interpet right ventricular ischemia in cases of pulmonary FE.

This presentation will impact the forensic science community by providing a useful tool for a better diagnosis of fatal pulmonary fat embolism.

FE is a common complication of blunt force injuries occurring in major traumas, especially if fractures of long bones are present. In cases of fulminating FE, the sudden massive obstruction of the pulmonary circle determines a rapid and often lethal increase in the impendence to right ventricular ejection with subsequent right heart ischemia and failure.

Recently proposed was a method to evaluate the occurrence of prevalent right ventricular ischemia determining acute right heart failure in cases of severe pulmonary FE. This method allows the morphological diagnosis of primary right heart failure due to acute persistent pulmonary obstruction. The major limits of this work are bound to its retrospective character: in relatively few cases of pulmonary FE, immunohistochemical investigations were performed on available paraffin-embedded blocks of cardiac tissue, collected at autopsy on the basis of a routine sampling protocol without an extensive systematic investigation of different anatomical regions. The study presented here has different goals. First, the preliminary study was investigated to show a more consistent number of cases in a prospective protocol. Another area of intrest was to study whether right ventricular damage is homogeneously distributed in the different regions of the right ventricle in cases of severe fatal FE. Finally, the question of the role of this method as a potential tool in the improvement of the medicolegal diagnosis of fatal pulmonary embolism was addressed during this study.

In a prospective study 220 consecutive autopsy cases performed at the University center of legal medicine in Geneva (Switzerland) between July 2010 and March 2012 were investigated. In each case, eight cardiac regions (anterior, lateral and posterior wall of the right and the left ventricle, anterior and posterior part of the interventricular septum) were sampled and standard histology staining (hematoxylin and eosin, Masson's trichrome) were performed. Moreover, immunohistochemical reactions with the antibodies against Fibronectin and the terminal complement complex C5b-9 were performed. FE was determined by means of frozen sections of the lungs (one sample from each lobe was collected and investigated), the central nervous system (one sample from the cerebral and cerebellar cortex and from the pituitary gland was collected and investigated), and the kidneys (one sample from each organ). The frozen sections were stained by oil red O staining.

The slides were investigated by two different observers with final consensual evaluation. In case of discord, a third forensic pathologist gave his advice and was allowed the final decision. Classical histology signs of fresh cardiac damage such as hypereosinophilia, presence of contraction bands, myocytolysis, fragmentation of the cardiomyocytes, interstitial bleeding, and inflammatory infiltrates were systematically searched and classified into four degrees (abstent, weak, moderate, and severe). Similarly, the immunohistochemical reactions against the antibodies Fibronectin and C5b-9 were classified into four degrees (negative reaction, single cell reaction, group cell reaction, and diffuse reaction). The degree of FE was determined following the method proposed by Falzi.

In this presentation, the results of this study will be presented and the implications for the routine medicolegal investigations will be discussed.

Fat Embolism, Right Ventricle, Ischemia

G124 Acute Eosinophilic Myocarditis in a Churg-Strauss Syndrome Case Treated With Montelukast: Immunohistochemical Study to Explain Pathophysiology

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The goal of this presentation is to present an uncommon fatal case of acute eosinophilic myocarditis in a 18-year-old man with Churg-Strauss Syndrome (CSS) treated with Montelukast. A complete methodological forensic approach by means of autopsy, histological, and immunohistochemical examinations led us to the conclusion of for a CSS with massive cardiac involvement.

This presentation will impact the forensic science community by showing how the absence of specific symptoms of cardiac involvement in a multiorganic CSS in active phase can make an early diagnosis difficult. This case report illustrates the importance of early diagnosis of CSS and underlines the possible relationship between LRA and CSS and its etiopathogenetic mechanism.

CSS is a systemic vasculitis with multiorganic involvement and represents a rare disorder with an incidence between 1.3 and 6.8 cases per 1,000,000 patients per year. It evolves through a prodromal phase characterised by asthma and atopic allergies (rhinitis) and eosinophilic infiltrative disease, a second phase with eosinophilic infiltration of tissues, and a third phase (vasculitic) characterized by systemic vasculitis. Three main histological features characterize it: necrotizing vasculitis, tissue infiltration by eosinophils, and extravascular granulomas. All organs may be involved (peripheral nerves, lungs, skin), but severe gastrointestinal, renal, cardiac, and central nervous system manifestations are associated with a poor prognosis. The heart is the major target (16% – 50% of cases) and its involvement is the major cause of death in CSS (48%), usually associated with an ANCA-negative status. Cardiac disease includes myocarditis, coronary vasculitis, valvular heart abnormalities, congestive heart failure, and pericarditis. The aetiology and pathogenesis of eosinophilia and tissue damage is unknown. On a cellular level, a strong shift toward a Th2-like response with massive T-cell activation and IL-4, IL-13, IL-10, and IL-5 production is evident. CSS patients usually have high serum levels of IgE. In the active phase, high levels of eotaxin-3 (a chemokine-involved organ damage) were found. The diagnosis of CSS is problematic, because none of the disease features are themselves pathognomonic and the numerous findings may have presented and evolved over a period of years. There are six clinical criteria proposed by the American College of Rheumatology (asthma, blood eosinophilia greater than 10% on differential white blood cell count, mono or poly neuropathy, migratory or transient pulmonary infiltrates detected radiographically, paranasal sinus abnormality, biopsy containing a blood vessel with extravascular eosinophils) with four being necessary for CSS to be diagnosed. However, these criteria are not pathognomonic in the absence of histologically proven vasculitis (eosinophil-rich and granulomatous inflammation involving the respiratory tract and

necrotizing vasculitis associated with asthma and eosinophilia). There are two different phenotypes, one "vasculitic," with manifestations due to small-vessel vasculitis (purpura, mononeuritis multiplex, glomerulonephritis), and one "eosinophilic," in which organ damage mainly results from tissue eosinophilic infiltration (pulmonary infiltrates, cardiomyopathy). ANCA positive patients usually show a more vasculitic phenotype. Although CSS is a rare disease, the number of reports increased in the last two decades after Leukotriene Receptor Antagonists (LRA) became available. In fact, in recent years several studies have reported the possible relationship between use of LRA and CSS expression. However, whether these drugs have a direct pathogenic role remains controversial.

Reported is a case of an 18-year-old man who was found lifeless at home in the bathroom by his parents. During the night he presented sweats, pain, and numbness in the leg. The extended family hadn't a history of sudden death or atopic disease. Two years before rhinitis, sinusitis, eosinophilia, and, above all, asthma were compared and treated with corticosteroids, antibiotic and antileucotrienic therapy (montelukast). The prick tests were negative. Serologic and PCR tests were negative for respiratory viruses and for *Legionella*, *Chlamydia,* and *Mycoplasma*. ANCA blood test was negative; the serum concentration of IgE was high. The last CT scan revealed bilateral ground-glass nodular lung opacities.

A complete postmortem examination was performed. The internal examination revealed only a polivisceral congestion and pulmonary edema. The surface of the heart showed opacity of the epicardium. The heart had a normal shape, size, and weight. The coronary arteries were normal and without significant stenosis or thrombotic occlusion. The valves were not thickened. The myocardium was flabby and pale with scattered patches of red and yellow-gray discoloration. The histological examination of the heart revealed diffuse and extensive infiltration by eosinophils with extensive loss of myocardial cells, pericarditis, necrotizing eosinophilic vasculitis, including small and epicardial coronary arteries, and colliquative myocytolysis.

The lungs showed eosinophilic pneumonia, necrotizing vasculitis with intimal and medial infiltration by eosinophils and extravascular granulomas, consisted of epithelioid macrophages and giant cells around large necrotic centers in a densely packed area of eosinophils with presence of the specific granules and nuclear debris. These granulomas were surrounded by eosinophils. The kidneys showed scattered necrotizing vasculitis and infiltration by eosinophils. Other organs, including the brain, showed no lesions.

An immunohistochemical examination of heart, lung, and kidneys samples with antibody anti CD4, CD8, CD45, EMBP (eosinophil major basic protein), IL10, TNF-alfa, and anti eotaxin-3 was performed to confirm diagnosis. The death was attributed to acute heart failure due to acute eosinophilic myocarditis in CSS.

Churg-Strauss Syndrome, Eosinophilic Myocarditis, Montelukast Therapy

G125 Sudden Unexpected Death of a Teenager Due to Peripheral T-Cell Lymphoma

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After attending this presentation, attendees will learn of an unusual hematological malignancy that caused the sudden and unexpected death of a 13-year-old girl, who had no prior clinical history or diagnosis of the condition.

This presentation will impact the forensic science community by highlighting a previously unreported cause of sudden, unexpected death of a teenager. This presentation will include a brief review of the classification schemata currently used by hematologists and oncologists for these type of malignancies and cluster differentiation markers used to establish the diagnosis.

The decedent, a 13-year-old female, was having a sleepover at the home of friends, whom she and her parents knew from church. At 2:00 a.m., she reportedly complained of chest and back pains and began to vomit. She requested the host family call her parents, as she wished to go home. Her mother and stepfather rushed to the place where she had her night out with friends. By the time her parents made the 12.5-mile 25 min trip, she had vomited several times and had become unresponsive. She was rushed to the hospital, where she was pronounced dead. During an in-depth forensic investigator interview, it was later revealed that she had complained of episodic chest pains for approximately two months. The pains were described as spasmodic chest and back pains, which started and resolved spontaneously. She was otherwise a normal child coping with scholastic challenges and playing soccer as part of her athletic curriculum. Her older male sibling had a childhood heart murmur, details unknown, which had resolved with age. Her biological father also had a heart murmur, not otherwise specified.

At autopsy, the decedent was of appropriate build and nutrition. Her heart at initial dissection revealed a thickened aorta when it was cut proximal to the arch. On further examination, the aorta was thickened at its origin, with extreme narrowing of the coronary ostia. The thickening also involved the pulmonary artery and the left atrium. Within the left atrium, the tumor had eroded the myocardium creating a 1cm defect in the anterior leaflet of the mitral valve, near the valve annulus. Histological sections revealed sheets of small-to intermediate-sized blue cells encasing the aorta, parts of the pulmonary artery, the left auricle and the mitral valve. The initial differential diagnosis suggested a Ewing sarcoma/primitive neuroectodermal tumor, lymphoma, or a rhabdomyosarcoma. The blue cells were strongly reactive to CD 45 and CD 3 with focal reactivity to CD 20, CD 99, and BCl-2. The tumor was diagnosed as a Peripheral T-Cell Lymphoma (PTCL).

The sudden, unexpected, death of an apparently healthy teenager is always tragic. Epidemiological studies on deaths in children under 20 years of age has a bimodal distribution with a large cluster under the age of 4 years and another large cluster in the 14 to 20-year-age groups. Much has been published about the causes of death in these two groups. A preliminary search of the English language literature revealed no cases of sudden, unexpected deaths due to hematological malignancy in the 6 to 14-year-age groups. In the United States, of the malignant lymphomas, the T-cell sub types are much fewer than those bearing B-cell markers. A British survey based on the tumor registries of England, Scotland, and Wales over a 20 year period identified 25 cases of T-cell lymphomas, comprising 1.6% on Non-Hodgkin's lymphoma registrations. PTCLs are extremely rare in children and very little is known about their natural history, therapeutic options, or prognostic indicators.

Sudden Death, Teenagers, Lymphoma

G126 Motor Vehicle Crash Involving an Elderly Woman With Undiagnosed Giant Cell Myocarditis

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After attending this presentation, attendees will be able to debate proposed National Association of Medical Examiners (NAME) standards for investigations of motor vehicle crash fatalities and be able to identify the histologic pattern of giant cell myocarditis.

This presentation will impact the forensic science community by discussing some of the controversy surrounding creation of autopsy performance standards for investigation of motor vehicle crashes.

A 92-year-old woman was traveling down a residential street when her vehicle impacted a maintenance vehicle on railroad tracks

that crossed perpendicular to the road. No evasive action or braking was noted. Externally, she had obvious long bone fractures of the lower leg and forearm, but no other potentially lethal external trauma was noted.

With recent proposed changes to autopsy performance standards from NAME regarding autopsies of drivers of motor vehicle crashes where no external trauma to account for death is present, an autopsy was conducted. At autopsy, internal exam revealed a large pericardial sac laceration and a right atrial laceration with an associated 1200ml. right hemothorax and right-sided rib fractures. Medical disease apparent on gross examination was significant only for mild coronary arterial atherosclerosis and no acute gross anatomical findings to account for death other than the injuries.

Per recent proposed changes for the NAME histological examination autopsy performance standard recommending histological exam when no cause of death is forthcoming on gross or toxicological exam or based on circumstantial evidence, submission of tissue for microscopic examination would not have been necessary. Because of the academic nature of this institution; however, histology is routinely performed on all major organs including the heart in the vast majority of autopsies. Unexpectedly, histological examination of the heart revealed a diffuse, multifocal, mononuclear inflammatory infiltrate with abundant eosinophils and rare, poorly formed granulomas with multinucleated giant cells within the interstitium, focally migrating into the surrounding myofibrils within the interventricular septum and left ventricle. Conversations with the next of kin disclosed that the decedent had been diagnosed with myasthenia gravis within the preceding few years. The histologic and circumstantial evidence was consistent with giant cell myocarditis.

Mononuclear and granulomatous myocarditis is a subset of inflammatory ailments of the heart that includes but is not inclusive of toxic myocarditis, infectious etiologies including tuberculosis, fungal and parasitic etiologies, sarcoidosis, autoimmune disorders, and hypersensitivity reactions.[1] In particular, giant cell myocarditis has been associated with intractable ventricular tachycardia and other arrhythmias and tends to occur in younger people with an average age of onset of 42.6 years +/-12.7 years.[2,3] A very extensive review study of granulomatous inflammatory disorders of the heart at autopsy uncovered the importance of giant cell myocarditis in the medicolegal arena in cases of apparent sudden death, especially in a younger age group.[4] Giant cell myocarditis is often associated with numerous autoimmune disorders including type 1 diabetes, systemic lupus, rheumatoid arthritis, alopecia totalis, and myasthenia gravis often associated with thymomas.[1,5-7] Giant cell myocarditis tends to have more eosinophils with myocardial fiber necrosis and poorly formed granulomas compared with the histologic pattern of sarcoidosis while sarcoidosis tends to have prominent granulomas and more fibrosis.[4,8]

This presentation will impact the forensic science community by discussing some of the controversy surrounding creation of autopsy performance standards for investigation of motor vehicle crashes. The recent proposal by NAME regarding autopsies of decedents in motor vehicle crashes has not been without some controversy especially when adherence to a revised standard may diminish the coroners/medical examiners/pathologists' discretion in triaging cases. Similarly, the suggested changes in the NAME standard for histological examination of tissues have also generated much discussion. As this case demonstrated, even adherence to the most comprehensively worded standards may miss rare findings.

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Giant Cell, Myocarditis, Motor Vehicle Crash

G127 Fulminant Myocarditis in Disseminated Mucormycosis: A Rare Cause of Death From a Snake Bite

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After attending this presentation, attendees will be able to identify an uncommon case of death due to mucormycosis myocarditis as a complication of viperine snake bite in an adult man. A complete forensic approach was performed through autopsy, and histological examination, and correlation with the clinical findings revealed that the cause of death was due to complication arising from disseminated mucormycosis, a rare complication seen in snake bite victims.

This presentation will impact the forensic science community by showing that disseminated systemic mucormycosis is a dreaded complication which can arise in many conditions, and can cause mortality, if left untreated.

Fungal infections are an important cause of morbidity and mortality. Mucormycosis is a ubiquitous filamentous saprophyte found in farms, gardens, forests, etc. and, unlike Candida and Aspergillus, it is generally not found in a hospital environment. The common portals of entry are respiratory, gastrointestinal tracts and skin. The outcome of mucormycosis is poor with 95% – 100% mortality in disseminated mucormycosis. Diabetes mellitus, especially diabetic ketoacidosis, high dose steroid exposure, neutropenia, degree of immunosuppression, renal failure, and poor hygienic conditions are some of the causative factors. Rarely, the disease has been reported in healthy people. Disseminated mucormycosis can produce fatal complications if it involves the cardiovascular system. Acute fulminant myocarditis is a critical clinical condition with sudden onset of severe congestive heart failure followed by severe hemodynamic deterioration. The case of a patient who developed acute fulminant myocarditis in the setting of disseminated mucormycosis secondary to snake bite envenomation is presented.

A 45-year-old male was bitten by a Russell's viper snake near the ankle of right leg while working in corn fields. He was transported to the hospital for treatment. In the hospital, he developed gangrene and compartmental syndrome in the region of snake bite, and wound debridement and fasciotomy was done as a part of management. He was treated with antibiotics as per the culture and sensitivity test reports. He developed acute arrhythmias and ST segment elevation was observed on continuous ECG monitoring. A 12-lead ECG showed concave ST elevation in almost all leads. The echocardiogram showed a normal left ventricular cavity without hypertrophy and preserved systolic function, no valvar vegetations, no pulmonary hypertension, and no significant pericardial effusion. He

succumbed due to irreversible cardiac arrest 19 days after admission, while under treatment at the hospital.

Postmortem examination revealed infected fasciotomy and debridement wounds with foul-smelling exudates. Multiple reddish circular haemorrhagic skin lesions of varying sizes from 5 – 11cm in diameter were present over the front and back of the trunk limbs. Epicardial and sub-endocardial hemorrhage was present involving the entire heart. Coronaries were patent. Hemorrhagic lesions were present in the greater omentum. An eroded necrotic area measuring 9cm x 8cm was present in the greater curvature of stomach. Hemorrhagic areas were present over the small and large intestines, kidneys, liver, and pancreas. Histopathological examination of the heart, kidneys, liver, and lungs showed irregularly shaped, non-septate hyphae with right angle branching with evidence of arterial invasion.

The cause of death was concluded as complications secondary to disseminated mucormycosis in a case of snake bite. Fatal fungal infections like disseminated mucormycosis can occur following snake bite envenomation. The physician should always keep this as a differential diagnosis and anticipate the fatal complications. Instituting early left ventricular support may improve outcome and result in better long-term survival. The forensic pathologist should differentiate the hemorrhagic cutaneous lesions from contusions and see evidence of fungal infections and their fatal affects during autopsies.

Myocarditis, Mucormycosis, Snake Bite

G128 A Sudden Death in a Fatal Case of Pneumococcal Overwhelming Postsplenectomy Infection Syndrome

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The goal of this presentation is to show how Overwhelming Postsplenectomy Infection (OPSI) syndrome is a rare condition, associated with high mortality. Gross examination, histological and immunohistochemical staining, and microbiological investigation are complementary to correctly gain a diagnosis.

This presentation will impact the forensic science community by showing the rarity of a pathological condition related to a post-traumatic splenectomy in a young man as well as the difficulty of gaining a complete study such as the one proposed. Autopsy, second-level histopathological and immunohistochemical studies, and microbiological tests helped forensic pathologists diagnose the pathological condition and exactly investigate cause of death.

OPSI is a rare condition and is a low-incidence entity with a high mortality rate despite aggressive therapy. Although initial symptoms may be mild and nonspecific, it can progress rapidly to Waterhouse-Friderichsen syndrome with full-blown septic shock and Disseminated Intravascular Coagulation (DIC). The incidence of serious infections after splenectomy remains low, with the incidence of fulminant OPSI ranging from 0.1% to 8.5%. Splenectomized patients are a significant infection risk, because the spleen has the largest accumulation of lymphoid tissue in the body. The initial presentation may be mild and non-specific but may rapidly progress to septic shock with DIC and Waterhouse-Friderichsen Syndrome (WFS). The mortality rate for OPSI has been estimated as approximately 50% to 70%, despite aggressive therapy. Of those patients who die, greater than 50% die within the first 48 hours of hospital admission. The mechanism that connects splenectomy to WFS is unknown but OPSI possible causes are loss of splenic phagocitary function, the decreasing IgM serum levels, a possible suppression of lymphocytes sensibility, and changes of opsonine's system. In this system, shocks irreversibility caused by endotoxin-like phenomenon of Sanerelli–Shwartzman. A case of rapidly progressive fatal overwhelming pneumococcal septicaemia and DIC with bilateral adrenal hemorrhages after splenectomy is reported.

Presented is a fatal case of a 32-year-old man admitted to the emergency department with a history of a few hours low-grade fever and non-specific symptoms (abdominal pain, diarrhea, nausea, and vomiting). On physical examination, not neurological, the body temperature was 39°C (orally recorded), blood pressure was 60/40mm Hg. A splenectomy after car crash was recommended. Tachypnea with oxygen saturation of 94% on room air was also recorded. He complained of mild abdominal tenderness and positive bowel sounds. Laboratory tests revealed leukopenia (WBC 2300), thrombocytopenia (PLT 66.000), metabolic acidosis, and disseminated intravascular coagulation with multiple organ failure. Chest X-ray showed emphasizing design bundling of bronco vascular pattern in the basal right pulmonary. Diagnosis of "abdominal colic" was performed. Death suddenly occurred less than five to six hours after admission. A complete postmortem examination was performed the day after death. Autopsy findings included widespread visceral petechiae, encephalic vascular congestion and cerebral oedema, pulmonary oedema with haematic fluid on the main bronchi. Bilateral acute hemorrhagic necrosis of the adrenal glands as like WFS was also detected. Alveolar necrosis, interstitial chronic phlogosis, hemosiderophages, and perivascular edema were detected at histopathological and immunohistochemical analysis as well as parenchymal complete apoplexy of adrenal glands. Postmortem microbiological investigation on blood, lungs, and vitreous humor were positive for Streptococcus pneumonia. Pneumococcal post-splenectomy infection syndrome rapidly overwhelming in septic shock and multiple organ failure in a young man affected with WFS was established as the cause of death.

OPSI, Waterhouse-Friderich, Splenectomy

G129 Fatal Progressive Disseminated Histoplasmosis Presenting as FUO in an Immunocompetent Italian Host

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The goal of this presentation is to focus on a fatal case of undiagnosed disseminated histoplasmosis occurring in an immunocompetent Italian host. A forensic approach by means of autopsy, microscopic examination, and microbiological studies led to the conclusion that the cause of death was septic shock caused by *Hstoplasma capsulatum* with pulmonary origin and overlapping of other fungal species infection.

This presentation will impact the forensic science community by demonstrating how important a thorough forensic investigation is to reach the correct postmortem diagnosis as well as showing that histoplasmosis could be an emerging sporadic infection in Western countries which needs to be addressed in clinical differential diagnosis in order to avoid unexpected death.

Histoplasmosis is a relatively rare infectious disease endemic in certain moist areas, such as East Africa, eastern and central United States, western Mexico, central and South America, and is mainly related to contact with soil containing feces of birds and bats. Most cases (95%) are acute, self-limited, and completely asymptomatic. Disseminated histoplasmosis has been described almost exclusively in immunocompromised hosts and in AIDS patients. Indeed, histoplasmosis is generally caused by a primary pulmonary lesion with a possible subsequent sporadic hematogenous dissemination.

Presented is a case of a 43-year-old Italian woman, previously splenectomized (due to the complications of a road accident) but

clinically immunocompetent, who was admitted to a regional hospital with a Fever of Unknown Origin (FUO) for several days. Two weeks later, in August, under suspicion of a lymphoproliferative disease, she underwent mediastinoscopy with incisional biopsy which revealed the absence of neoplastic lesions and specific granulomatous structures. Approximately one month later, following the appearance of hepatomegaly with altered indices of cholestasis (without jaundice) and the persistency of fever, the patient underwent liver biopsy with the sole evidence of a necrotizing granulomatous inflammation. Finally, ten days later, a bone marrow biopsy was suggestive for necrotizing inflammatory lesions of infectious etiology. Due to the rapid onset of sepsis, a sudden deterioration of general conditions occurred and the patient died.

A forensic autopsy was performed within 48 hours after death in order not only to find the real cause of death but also to investigate the hypothesis of medical liability (delay in diagnosis, lack of adequate therapy, and so on) of the clinician who had taken care of her.

On the basis of internal examination (sectioning the lung, an apparently capsulated blackish round lesion—1.5cm in diameter—appeared; also a reddish-brown focus with a yellowish central area was found. Other multiple dense spots in the pulmonary parenchyma were observed) and evaluation of histological specimens obtained from autoptic samples (jeweled oval cells of 1 – 5 microns in diameter), it was possible to formulate a diagnosis of histoplasmosis with polivisceral localization (lung, liver, brain, bone marrow), with overlapping—in the lungs—of other fungal species infection (Candida sp.) and arterial embolization of hyphae and pseudo-hyphae.

The diagnostic difficulties due to the inability to document a histoplasmosis in the early stages of the disease, more so in any clinical-radiological pictures which were not sufficiently specific and consequently clinically subtle, led to the accused clinicians being found not guilty of manslaughter. However, this case underlines the need to keep attention levels high in the clinical and forensic analysis of all the granulomatous lesions of unclear etiology which require histological and microbiological studies of blood and infected organs as well as detection of antigens in blood or urine samples by ELISA or PCR.

Fatal Histoplasmosis, Immunocompetent Host, Histopathology

G130 The Application of Forensic Microbiology in a Fatal Case of Transfusion-Transmitted *Yersinia Enterocolitica* Sepsis

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The goal of this paper is to underline the usefulness of the use of forensic microbiological analysis to explain the cause of death in case of infective disease.

This presentation will impact the forensic science community by presenting the rare case of septic shock due to transfusion-transmitted *Yersinia enterocolitica*. The use of postmortem microbiological investigation on cadaveric blood gives support to the forensic pathologist to diagnose sepsis-related death. In this case, the forensic microbiological analysis allows the exact correspondence between the pathogen agent isolated from transfused Red Blood Cells (RBCs) and the etiological agent that determined the fatal sepsis, *Yersinia enterocolitica*.

This presentation concerns the fatal case of a woman infected by an RBCs transfusion bag contaminated with *Yersinia Enterocolitica*. *Yersinia enterocolitica* is a pleomorphic gram-negative bacillus that belongs to the family Enterobacteriaceae and causes mild disease, most frequently acute diarrhea, terminal ileitis, mesenteric lymphadenitis, and pseudoappendicitis. *Yersinia enterocolitica* most commonly affects young individuals, but whether this represents an increased susceptibility or a greater likelihood of developing symptomatic illness is unclear. Human yersiniosis is attributed to contaminated pork, milk, water, and tofu consumption, as well as blood transfusion. Bacterial sepsis has become the most frequent infectious complication of transfusion, but cases of transfusion-transmitted *Yersinia enterocolitica* sepsis with bacterial isolation are very rare in postmortem examination.

A 37-year-old woman, pregnant with her second child, was admitted to the gynecology and obstetrics unit with diagnosis of post-term pregnancy (41+3 weeks of pregnancy). The course of pregnancy was physiological. The fetus was in cephalic presentation, the amnio-chorial membranes were intact. On the third day of hospitalization labor was induced following amniorrhexis and, in the absence of contractile activity, the oxytocic perfusion was started. The patient responded positively to the oxytocic perfusion and gave birth to a female child with eutocic parturition. The prophylaxis of postpartum hemorrhage was made by administering of oxytocin and methylergometrine, and the suture of a vaginal laceration was performed. The woman, after the parturition, was affected by a hemorrhagic postpartum shock determined by trans-vaginal blood loss estimated at 1800ml during and after the parturition. Blood chemistry and blood count laboratory tests were performed and showed reduction of Hb, Hct (Hb 5.9, Hct 18.6) with a condition of acute anemia. On the fourth day of hospitalization (first postpartum day), the patient was transferred to the intensive care unit for the serious anemic condition; serial blood chemistry and blood count laboratory tests were performed and fresh frozen plasma and RBCs bags were transfused. The patient's clinical condition worsened and she showed a state of shock refractory to therapy, kidney failure, and CID; on the fifth day of hospitalization (second postpartum day), she was admitted to the operating room for an abdominal laparotomy and hysterectomy leaving uterine adnexa *in situs*. In the following days, the woman showed hemodynamic instability, respiratory distress despite the support with vasoactive amines, and mechanic ventilation. On the eighth day of hospitalization (fifth postpartum day), she died of septic shock. A complete postmortem examination was performed 24 hours after death and showed surgery outcomes of hysterectomy. Subpericardial and subpleural petechiae were detected. The other organs did not show specific alterations except for intense vascular congestion and generalized edema. Histological examination showed in lung specimens alveolar septa mildly thickened by edema and capillary congestion, alveolar edema, hyaline membranes lining the denuded alveolar walls, alveolar infiltrates of polymorphonuclear neutrophilic leukocytes, pigmented macrophages, monocytes and plasma cells, alveolar hemorrhages; in kidney specimens acute tubular necrosis and wide hemorrhages. In all organs, in particular in the lungs, small vessels contain fibrin thrombi. The cause of death was a fatal septic shock. On blood samples collected during the autopsy a microbiological analysis was performed using PCR to isolate pathogens responsible for septic shock. PCR analysis showed positive for *Yersinia Enterocolitica*. After the isolation of *Yersinia Enterocolitica* from the dead woman's blood sample, the infected transfused RBCs were identified. High titers of antibodies against *Yersinia Enterocolitica* were detected in the donor's plasma sample one month after blood donation. The donor had no clinical signs of intestinal infection at the time of donation.

Transfusion-Transmitted, Forensic Microbiology, Septic Shock

G131 Routine Postmortem Cultures in Infant Autopsies: A Four Year Experience

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After attending this presentation, attendees will determine the value and effectiveness of learning routine postmortem cultures on infant autopsies and appreciate the importance of correlating postmortem culture results with gross and microscopic autopsy findings.

This presentation will impact the forensic science community by improving knowledge of the utility of postmortem cultures and by redefining roles of routine postmortem cultures on current protocols for infant autopsies.

A comprehensive infant autopsy not only includes an external and internal examination, but also many ancillary diagnostic tests including postmortem cultures, radiology, toxicology, histology, and metabolic studies. Each of these components may contribute to some degree in determining a cause of death. The purpose of this study is to specifically examine the relative contribution of routine postmortem cultures in establishing a cause of death in infant autopsies.

Postmortem cultures have long been regarded as an area of contention in the field of forensic/autopsy pathology and microbiology. There is no doubt that postmortem cultures not only provide epidemiological information for public health purposes but also play an integral role in helping to establish a specific cause of death in some cases. However, due to postmortem contamination, postmortem cultures can be difficult to interpret.

Bacterial cultures of blood, lung, spleen, and cerebrospinal fluid as well as viral cultures from the respiratory tract are routinely collected using sterile instruments on the majority of infant autopsies at the Harris County Institute of Forensic Sciences. The results of routine postmortem cultures from 304 infant autopsies ranging from 1 to 365 days of age over a four year period (2008 – 2012), presented with sudden unexpected deaths of previously healthy infants and without gross anatomic abnormalities or evidence of infection were analyzed. Data is available fpr 295 blood cultures, 299 lung cultures, 290 spleen cultures, 255 cultures of cerebrospinal fluid, and 238 viral cultures.

The data shows that the cause of death was determined to be infectious in 12 out of 304 pediatric autopsies (4%). In these autopsies with an infectious cause of death, eight of these cases had a positive postmortem culture and histologic correlation with the cultured organism; four had a negative postmortem culture with histologic evidence of infection. Out of autopsy cases with an infectious cause of death, five of the 12 viral cultures from the respiratory tract and none of the bacterial cultures of cerebrospinal fluid successfully isolated an organism that contributed to the cause of death.

Two hundred and twenty-one out of 304 pediatric autopsies (73%) were classified as sudden infant death syndrome or undetermined (co sleeping); 157 of these cases had a positive postmortem culture without histologic evidence of infection (postmortem contamination). The postmortem interval, like previous studies have shown, did not have a significant impact on the results of the postmortem cultures.

In conclusion, routine postmortem cultures will fail to identify a definitive cause of death in a significant proportion of pediatric autopsies that present with sudden unexpected deaths of previously healthy infants and with no gross anatomic abnormalities or evidence of infection at autopsy. Lastly, the high postmortem contamination rate complicates the interpretation of postmortem culture results.

Culture, Infant, Autopsy

G132 Vertebral-Medullary Trauma Death: When, Where, and How Was the Trauma?

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After attending this presentation, attendees will receive information on how to manage cases with minimal signs at autopsy and hospital information on how to reconstruct a traumatic event in order to clearly assign the responsibility of trauma. A careful evaluation of all the medical documents is the cornerstone to understanding the traumatic mechanism and timing of vertebral injury, especially when autopsy findings are minimalized because of a post-traumatic surviving interval.

This presentation will impact the forensic science community by understanding how the clinical information can be of relevant importance in cases of a trauma death with minimal autopsy signs.

A 45-year-old man died of sepsis secondary to quadriplegia due to complex multiple fractures of the lower cervical column (C4 - C7) after a three-week hospitalization in the intensive care unit.

The family, interviewed before performing the autopsy, declared that the man had been arrested for an alleged fight with a neighbor the day before. They reported that when the statement was taken in the police station, the police officer noticed a strange, irascible behavior and a neuropsychiatric consultation was requested.

The medical documentation shows that the man was hospitalized in the psychiatric unit and suddenly some neurological signs appeared. Then the patient was moved to the orthopedic unit and checked radiologically with a positive result of an instable C5 fracture, and a Philadelphia collar stabilizer was applied.

His condition rapidly worsened and he was transferred to the intensive care unit with respiratory arrest, peripheral and central cyanosis, and fixed and unreactive pupils (GCS=3). The blood pressure and oxygen's saturation were undetectable and the pulse was 40bpm. He was reanimated, intubated and a cardiotonic was administered; the patient recovered cardiorespiratory function (pulse 120 – 130bpm). Complex monitoring, sedation, and algotherapy with morphine followed.

After some days, an extensive CT scan of the head and neck was performed and no pathological or traumatic signs were found in the head, but multiple fractures from C4 to C7 (fractures of the spinous process of C4 and C5; fractures of the vertebral bodies of C5 (type III), C6 (type I), C7 (type I) were detected. After ten days of admission in the intensive care unit, the patient presented convulsion and fever (BT: 38.7°C).

During hospitalization the patient received fluid supply, human albumin 20%, mannitol 20%, antibiotic therapy according to antibiogram, antiaggregant, barbituric, analgesics, sedatives, antiacid and vitamins. Despite this intensive treatment his hemodynamic state was unstable and his fever rose to 40.9°C and biochemical signs of multiple organ failure appeared (leukocytosis $15x10^9$/L, AST 249 IU/L, ALT 580 IU/L, hypoalbuminemia, urea 11.2mmol/L, glucose 36mmol/L). The patient died after 23 days of intensive care with diagnose of sepsis.

At autopsy, the external examination revealed two decubitus areas, in the occipital and sacral area with skin necrosis, marks of defibrillation and no signs of trauma. The classical autopsy opening procedure was completed with a complete posterior neck dissection discovering a minimal, insignificant paravertebral blood infiltration on the C4 - C5 area, and the cervical column was taken away for further bone and spinal cord evaluation. The internal organs showed macroscopical signs of failure and sepsis. The spinal cord, fixed in formalin, presented macroscopically an external, light brownish discoloration investigated microscopically. The cervical vertebrae were prepared in order to observe the localization, type, and state of the fracture's repair process.

The forensic evaluation of the case confirmed a linear correlation between the vertebral-medullar injuries and the death. The CT images revealed clear, multiple complex fractures in the cervical area that could only be the result of a relevant neck trauma.

The localization of the multiple vertebral fractures in the lower cervical spine allows the existence of a free interval of neurological signs, and the instability due to the complexity of the fractures (C5) can explain the sudden deterioration of the neurological condition as the result of small, ordinary neck movements.

The big forensic questions to answer concern the moment and the mechanism of the vertebral trauma, because many different situations and persons could be involved in this case (the discussion/fight with the neighbor, abuse of force by the police, the event which occured in the psychiatric unit, fall, use of restraints,

altercation with another person) or a traumatic event before the arrest.

Spinal Cord Trauma, Forensic Pathology, Cervical Fractures

G133 A Single Case of Natural Death Due to Idiopathic Hypereosinophilic Syndrome Occurring in a Young Nigerian Prostitute

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After attending this presentation, attendees will be able to better understand the main pathological features of Hypereosinophilic Syndrome (HES) found at autopsy in a 21-year-old Nigerian prostitute.

This presentation will impact the forensic science community by discussing the possibilities of natural death caused by HES in a young prostitute. The place and circumstances in which she was found initially led to a supposition of a violent death.

In February 2012, early in the morning, a 21-year-old partially undressed Nigerian prostitute was found dead next to a rubbish bin, in a central square of Palermo, Italy.

After the external examination, rectal and vaginal swabs were taken for genetic analysis in order to find sperm. After a complete autopsy, organ samples were also taken for histopathological examination, using routine histological stainings, namely hematoxylin and eosin, Giemsa and PAS. Toxicological and serological investigations and hematological analysis were performed as well.

The external examination and autopsy didn't reveal any traumatic lesions or signs of sexual abuse as confirmed by the genetic investigation, which was negative in the research of spermatozoa. The serological analysis was also negative for viral and parasite infections and the toxicological investigation was negative for drugs and alcohol. The blood sample taken two hours after death showed that the white cell count was 9500/mm^3 with 2840 eosinophils/mm^3 (41%). The histopathological investigation showed: (1) lungs: marked eosinophils recruitment and degranulation with endovascular endothelial injury and formation of occluding platelet plugs; multi focal vasculitis with eosinophilic infiltrate and intimal myofibroblastic eccentric thickening; (2) heart: marked congestion and subendocardial myocytolysis and interstitial eosinophilic infiltration; (3) lymph nodes: normal structural organization with eosinophilic granulocyte infiltration of lymphatic sinuses; (4) liver: cloudy swelling of epatocytes which show granular cytoplasm; focal eosinophilic infiltration of fibrotic and thickening portal spaces; (5) kidneys: diffuse and marked vascular congestion and cloudy swelling of cortical convoluted tubules; marked and diffuse eosinophilic infiltration of renal pelvis; and, (6) bone marrow: normal hematopoietic components with prevalence of eosinophilic granulocytes in the myeloid series.

HES is a rare disorder characterized by a sustained overproduction of eosinophils, peripheral eosinophilia, and tissue eosinophilic infiltration. It commonly affects the heart, lung, skin, and central and peripheral nervous systems, and often causes impaired organ function.

Until the late 1990s, this disease had a bad prognosis with a median survival of less than one year with less than 20% of patients surviving two years, and with death usually occurring because of organ dysfunction. Current treatment has fortunately improved the prognosis.

In this case, however, because of the fact that this young prostitute lived *underground*, no information on the clinical development of the pathology which led to exitus exists and also the absence of any medical treatment didn't allow the course of the illness to be slowed down. As the hematological examination is from a single sampling which ran for approximately two hours after death, it is not possible to document the duration of this marked hypereosinophilia, the histopathological examination showed a reacutization of chronic eosinophilic vasculitis responsible for acute respiratory failure, the cause of death. In this case, the absence of a specific etiology has allowed us to define the HES as idiopathic.

Hypereosinophilia, Histophat Findings, Autopsy

G134 Medium-Chain Acyl-CoA Dehydrogenase Deficiency: A Differential Diagnosis in a Patient With Mental Status Changes Suspected of Drug Toxicity

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The goal of this presentation is to show how rare inborn errors of metabolism such as Medium-Chain Acyl-CoA deficiency can be present with symptoms commonly associated with drug toxicity.

This presentation will impact the forensic science community by showing how patients with nausea, vomiting, and acute mental status change in the setting of fasting should be tested for inborn errors of metabolism such as Medium-Chain Acyl-CoA deficiency if toxicology is negative. When recognized, family counseling can be initiated and additional lives potentially saved.

This case involves a 30-year-old white male coal miner who experienced nausea and vomiting with a 30-pound weight loss over the last three months of his life. He was admitted to the local hospital for a further evaluation of intractable vomiting and complaints of abdominal pain and scant hematemesis. Upon admission, the patient appeared to be in no acute distress and was alert and oriented to person, place, and time. Admission laboratory investigation demonstrated blood glucose of 71mg/dL (70 – 99), BUN 31mg/dL (7 – 18), Creatinine 2.0mg/dL (0.6 – 1.3), and an anion gap of 23.7mEq/L (10 – 20). Several hours into the admission, the patient became confused, anxious, and appeared to be hallucinating with bizarre manifestations, such as licking the air followed by self-injurious behaviors such as banging his head on the wall and trying to jump out a window. He was subsequently placed in soft restraints for his own and others' safety. Fifteen minutes later the patient became unresponsive and developed a rapid respiratory rate (40/min). In another 15 min, respirations and cardiac electrical activity suddenly ceased, and resuscitation attempts were unsuccessful. No ventricular fibrillation was observed during his hospitalization. Drug toxicity, specifically "bath salts," were suspected due to the patient's mental status changes, a history of remote drug abuse in the patient's past, and the prevalence of psychosis-inducing drugs in the community. Drug testing for synthetic stimulants such as mephedrone, MDPV, and methylone were all negative. The past medical history of this patient was unremarkable for previous mental status change. He furthermore had a sibling who had experienced a mild mental status change during a fast for colonoscopy, which was relieved with electrolyte drink and broth.

On autopsy, the patient was a well-developed male with features suggestive of recent weight loss (loose soft abdominal skin and striae). Findings included gross and microscopic microvesicular steatosis, mild cerebral edema, and subnuclear vacuoles of the renal cortical tubules. Autopsy urine and blood were negative for ketones. Postmortem screening of the blood, urine and vitreous were positive only for ethanol and promethazine prescribed in the hospital. Due to the odd presentation of fatty liver, negative drug screen, vomiting, and altered mental status, the forensic pathologist elected to request postmortem metabolic screening of the blood and bile, which was

positive. The patient was found to have died due to acute metabolic decompensation caused by Medium-chain acyl-CoA dehydrogenase deficiency, an inherited fatty acid oxidation disorder. Discussed will be the necessity of awareness of such entities as adult manifestation of inborn errors of metabolism in spite of their relative rarity.

MCAD Deficiency, Mental Status Change, Metabolic Disease

G135 Postmortem Distribution of 3-Beta-Hydroxybutyrate

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After attending this presentation, attendees will understand how metabolic and biochemical disturbances existing at the time of death and potentially leading or contributing to death can be better characterized by 3-beta-hydroxybutyrate (3HB) determinations in blood and in biological fluids such as urine, vitreous, pericardial, and cerebrospinal fluids.

This presentation will impact the forensic science community by demonstrating how simultaneous determinations of 3HB in blood, urine, vitreous, pericardial, and cerebrospinal fluids may provide useful information pertaining to the duration of the death process. Indeed, the equilibrium between blood and other body compartment fluids (especially vitreous, pericardial, and cerebrospinal fluids) is established over time. The presence of comparable vitreous, pericardial, and cerebrospinal fluid 3HB values to blood 3HB values can thereby confirm that metabolic and biochemical disturbances and the death process developed over several hours. Additionally, this presentation will impact the forensic community by demonstrating that cerebrospinal fluid 3HB levels exceeding 2000µmol/l can be regarded as biochemical markers of concomitant, pathological blood 3HB increases, thus allowing metabolic and biochemical disturbances leading to death to be reliably diagnosed.

A total of 158 subjects were selected for this study. Case inclusion criteria were postmortem interval, circumstances of death, and availability of all biological fluids upon autopsy. After having performed postmortem investigations including native CT scan, autopsy, histology, biochemistry, and toxicology, 12 fatal diabetic ketoacidosis cases and eight free-ethanol hypothermia fatalities were identified. Furthermore, five other cases concerning sudden deaths in subjects presenting chronic alcohol abuse and no previous diagnosis of diabetes mellitus according to medical records were included, as well as 20 bodies presenting mild decompositional changes with all biological fluids upon autopsy. According to the medical records, all these cases were non-diabetics. All biological samples were transferred to the laboratories immediately after collection. When analyses were delayed, biological samples were stored at -20°C. Biochemical investigations including blood acetone, vitreous glucose, blood glycated hemoglobin as well as blood, vitreous, urine, pericardial and cerebrospinal fluid 3HB determinations were systematically performed.

The results of the study indicated that vitreous and pericardial fluid 3HB levels were reliable markers of underlying metabolic disturbances leading to death in diabetic ketoacidosis cases. In hypothermia fatalities, 3HB concentrations in pericardial fluid were the most representative compared to those in blood. In bodies presenting mild decompositional changes, vitreous and pericardial fluid 3HB levels were the closest to blood levels. Lastly, in sudden deaths related to chronic alcohol consumption, vitreous and pericardial fluid 3HB levels were the closest to blood 3HB concentrations.

Increases in blood 3HB concentrations seem to be reflected in parallel increases in pericardial fluid 3HB levels, irrespective of the cause of death. Vitreous humor can be considered a further, alternative, biological substrate for 3HB determination. Conversely, the interpretation of urine 3HB levels in diagnosing pathologically significant ketoacidosis requires more cautiousness. Lastly, markedly increased cerebrospinal fluid 3HB levels (over 2000µmol/l) can be considered a reliable indicator of underlying metabolic disturbances potentially leading to death and can be used to diagnose pathologically significant ketoacidosis.

Forensic Sciences, Distribution, Beta-Hydroxybutyrate

G136 A Rare Case of a Large and Bilateral Acute Cerebral Necrosis Following a Very Low Total Dose of Conventional Radiotherapy Treatment for Anaplastic Oligoastrocytoma

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The goal of this presentation is to examine the histopathological aspects of an acute cerebral radionecrosis following conventional radiotherapy at subtherapeutic dosages associated with adjuvant chemotherapy. The adoption of adjuvant chemotherapy may therefore increase the incidence of radionecrosis due to its radiosensitization effect.

This presentation will impact the forensic science community by showing the necessity of a complete methodological forensic approach by means of autopsy, histological, and immunohistochemical examinations to diagnose an unusual early cerebral radionecrosis, due to a very low dosage of conventional radiotherapy and to differentiate from tumor recurrence.

Malignant gliomas are the most common primary brain tumors, and glioblastoma multiforme and anaplastic oligoastrocytoma comprise the greatest part of these. The actual standard protocol of treatment for these patients consists in maximal safe resection followed by radiotherapy and concomitant and adjuvant chemotherapy (temozolomide). However, even with treatment, outcomes are poor. The median survival for patients with glioblastoma multiforme is 10 – 12 months, whereas two-year survival rates for glioblastoma multiforme and anaplastic astrocytoma are only 9% and 44%, respectively.

Radiation doses in the region of 46 – 50 Gy are as efficacious as higher doses in the treatment of low-grade glioma, but doses of 60 Gy provide better outcomes for high-grade gliomas and are commonly prescribed for these tumors.

Radiation therapy for the brain, however, may often result in several episodes of acute and chronic damage, even if the total dose of radiation appears to be the most important risk factor for subsequent necrosis. Three distinct periods of radiation effect may be identified: acute (during the radiation itself), early delayed (at around 1.5 months after the radiation), and late delayed (between 4.7 and 7.6 months and for more than two years). More simply, radiation-induced effects are considered late effects if they occur after 90 days from the first day of radiotherapy. However, necrosis has been reported as early as three months and as late as 47 years after radiotherapy.

Reported is a rare case of large and bilateral acute cerebral necrosis following a very low total dose of conventional radiotherapy.

A 56-year-old female first presented epileptic attack associated with aphasia and paralysis. Magnetic Resonance (MR) imaging showed a lesion of 2cm in diameter in the left temporal lobe, without perilesional edema and gadolinium enhancement after perfusion. The tumor was totally excised via a left fronto-temporal approach. The histological examination of the mass confirmed the typical Anaplastic Oligoastrocytoma. Following the operation, a treatment with a total dose of 60 Gy conventional radiotherapy was programmed, in 1.80 Gy daily fractions, associated with steroids (desamethasone, 8mg each day) and chemotherapy (temozolomide, 100mg each day). After the

twentieth dose (a total dose of 36 Gy), she presented an epileptic attack associated with fever, tremor, confusion, aphasia, and recurrent focal deficit. MR imaging showed a mass with edema in the fronto-temporal lobes which suggested radiation-induced necrosis, without enhancement by gadolinium. In the following month, the woman died and the autopsy was effectuated.

The external examination was unremarkable. The examination of the brain, regular in size and weight, after fixation in buffered formalin revealed diffusely swollen cerebral hemispheres. On coronal sections, the cerebral frontal and temporal lobes revealed regions of necrosis and cortical hemorrhages.

The etiopathogenetic definition was outlined by histological examinations performed on brain tissue samples using Haematoxylin-Eosin (H&E) and Masson and it revealed the presence of diffuse and marked cytotoxic and vasogenic brain edema, and in samples taken from right and left fronto-temporal lobes foci of central necrosis and extensive cortical hemorrhages, marked stasis, and cerebral vessels surrounded by inflammatory cells, above all granulocytes. The immunohistochemical examination of the brain specimens was also conducted for antibodies anti-GFAP (glial fibrillary acidic protein), CD68, CD15, TNF-α, fibrinogen, HSP, cytokeratins, and TUNEL. The other organs showed signs of central dysregulation (pulmonary edema).

The exitus was attributed to an acute central dysregulation caused by edema and necrosis radiation-induced and relative complication with rapid increase of intracranial pressure.

In conclusion, according to scientific literature, the cerebral necrosis could appear in areas radiated to less than 50 Gy, but with daily fractions of 2.25 Gy. In this case, the cerebral necrosis was very large and it was caused by inferior dosage (36 Gy), so it corroborates that the adjuvant chemotherapy, temozolomide in particular, significantly increases the risk of cerebral necrosis by approximately fivefold. A review of the literature is presented

Cerebral Radionecrosis, Anaplastic Oligoastrocytoma, Immuhistochemistry

G137 Fatal Hemorrhage From a Tracheostomy Site in a Patient With Blue Rubber Bleb Nevus Syndrome: A Case Presentatio and Review of Literature

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After attending this presentation, attendees will understand the importance of a thorough examination of the viscera and soft tissues, especially in the head and neck region, and gain insight on potential complications including sudden death from the rupture of soft tissue vascular malformations due to emergency tracheostomies.

This presentation will impact the forensic science community by increasing the awareness of the rare Blue Rubber Bleb Nevus Syndrome (BRBNS)and the risk it presents for fatal hemorrhages depending on its location. A review of literature on the topic of fatalities linked with tracheostomies will be presented.

This case involves a 31-year-old Caucasian female with a medical history of multiple arteriovenous malformations on the right side of her body and a diagnosis of BRBNS. She went to the emergency room because of an infected tooth. Several years prior to her visit to the emergency room, she had had a single episode of bleeding-from-the-tongue-based AVM. Prior to extracting the tooth, she was injected with lidocaine and developed acute anaphylaxis with severe facial and airway swelling resulting in an obstructed airway. An emergency tracheostomy was performed, which was complicated by profuse hemorrhage from the adjacent ruptured soft tissue vascular malformation. Hemostasis could not be achieved by the Intensive Care Unit staff and the patient exsanguinated. The body was brought to the Office of the Cook County Medical Examiner for postmortem examination. An autopsy determined that the immediate cause of death was anaphylaxis due to a reaction to the anesthetic medication that was given prior to the tooth extraction; however, the condition, that significantly contributed to her death was hemorrhage due to a ruptured vascular malformation during the tracheostomy. The uncommon location of an exsanguinating vascular malformation in the neck region in a patient with BRBNS deserves a generous discussion.

Approximately 153 cases of BRBNS have been reported in the literature. What was possibly the first description of BRBNS appeared in 1860 in a paper by Dr. Gascoyne. Dr. Gascoyne described a nevus involving the parotid gland, which caused death from suffocation in a patient who displayed numerous nevi of the viscera. Dr. William Bean coined the name BRBNS in 1958 because of the rubbery, nipple-like texture of the cutaneous hemangiomas that tend to camouflage vascular malformations found in the viscera, and in this case, the neck region. BRBNS has since been reported in patients with numerous and varied types of cutaneous vascular nevi frequently associated with visceral malformations. Most commonly, these visceral malformations are located in the small and large intestines and cause death from hemorrhage. Reports of BRBNS associated with findings of hemorrhaging vascular malformations in the head and neck causing suffocation have also been reported. The association of BRBNS and hemorrhaging vascular malformations of the head and neck has led anesthesiologists to develop various techniques to manage the airway in these patients without disrupting the lesions. Although BRBNS is usually diagnosed in infancy, there are, unfortunately, cases of BRBNS which remain undetected until adulthood. A possible genetic inheritance pattern is currently being studied.

Cases describing prominent head and neck findings in patients with BRBNS emphasize that they often first present to an otolaryngologist or oral surgeon with the incidence of oral hemangiomas as high as 59% to 64%. None of these cases has discussed the risks and potential complications of emergency tracheostomy placement in these patients. This case is the first to discuss a patient with BRBNS who underwent a tracheostomy and developed a fatal hemorrhage due to this rare presentation.

Nevus, Tracheostomy, Hemangioma

G138 Toward Validating a Universal Equation for Estimating Postmortem Interval

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After attending this presentation, attendees will understand that it is possible to provide an accurate presumptive estimate of Postmortem Interval (PMI) within a few hours of attending a death scene.

This presentation will impact the forensic science community by demonstrating the use of a new method for medicolegal death investigators to gather information useful for making timely decisions on the scope of the investigation and the use of resources.

Estimating PMI is a critical step in every death investigation. During the initial interview process, a first responder/investigator will attempt to determine the time at which the deceased was last known to be alive. This information is not always helpful to establish a firm PMI. The information for a more reliable PMI can be critical to evaluate alibis, identify the deceased, establish a working hypothesis on the manner of death, lend weight to other information through corroboration, and corroborate the cause of death. Several methods have been developed to estimate PMI since the mid 19th-century, but the development of a universal method remains elusive. Two universal equations were recently proposed in the forensic science literature.[1] One of these equations was developed for above-ground death scenes and presents PMI as a function of soft tissue mass loss,

temperature, and relative humidity. In response to the need for validating this equation in other climates, we have begun the process of validating this equation in southeastern Nebraska, USA. This region is located in a cold climate characterized by hot summers and the lack of a dry season (climate code: Dfa).

Presented are the findings from three death investigations where the estimate generated by the above-ground PMI equation was used and compared to a known PMI. Temperature and relative humidity were measured at intervals of one minute with a datalogger placed as close as possible to the corpse. These measurements were collected for 90 to 150 minutes.

It is shown that this method has real time application for corroborating other information and thus making a more robust initial estimation of PMI. It is often the case that first responders and crime scene investigators need just one additional component of information to make a confident decision on the parameters of the investigation to confidently proceed with further investigative tasks such as interviewing witnesses. On the other hand, other information may not be available, or is unreliable, so that the method herein provides the primary information for estimating PMI. The current method is such that it can be taught easily to first responders and crime scene investigators. Supervisors and others who have an interest can easily check the data points by looking at digital images, collecting official weather data, and conducting the calculations of the method. This is a real-time viable tool that only awaits additional research and application.

The current results complement the universal equation for above-ground death scenes proposed in the recent literature. Results indicate that overall, the proposed equation was accurate in helping to narrow the time frame for an estimated PMI. Since the percentage of soft tissue loss is subjective, it is recommended that more than one individual observe the physical characteristics of the corpse to determine an average tissue loss. This procedure will allow for a more accurate range for tissue loss and minimize the error associated with estimating PMI.

Reference:

1. Vass AA. The elusive universal postmortem interval formula. Forensic Sci Int 2011;204:34-40.

Medicolegal, Taphonomy, Death

G139 Using Interpolation to Estimate Postmortem Interval on the Condition of Various Ambient Temperature

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After attending this presentation, attendees will learn how the ambient temperature influences the postmortem blood pH values and how to use interpolation to estimate Postmortem Interval (PMI) at various ambient temperatures.

This presentation will impact the forensic science community by enabling the forensic pathologists to better understand the correlation between blood pH values and various ambient temperatures at PMI. The relationship between blood pH values and ambient temperatures at different PMIs analyzed by interpolation function fitting provides another useful tool in the estimation of time since death.

The determination of the time since death is a primary task of medicolegal death investigation. Few accurate methods currently exist to evaluate the (PMI) at various ambient temperatures. Interpolation analysis was applied to study the correlation between blood pH values and ambient temperatures at different PMIs. A total of 48 rabbits were randomly divided into six groups and sacrificed by air embolism. Five mL of blood from the right ventricle were sampled immediately after death and then placed in sterile blood tubes. The blood specimens from each group were placed in water baths at 10°C, 15°C, 20°C, 25°C, 30°C. and 35°C temperatures, respectively. At different PMIs (once every four hours), the blood pH values were measured by meter electrochemical analyzer.

The results showed that there was a strong correlation between the blood pH values and PMI at various ambient temperatures. At different ambient temperatures, pH values decreased at different rate. Under temperatures of 10±0.05°C, it took 64h for the pH value to drop from 7.528±0.037 to 6.143±0.029. Under temperatures of 20±0.05°C, it took 38h for the pH value dropping from 7.528±0.037 to 6.141±0.035. Under temperatures of 30±0.05°C, it took 20h for the pH value to drop from 7.528±0.037 to 6.141±0.037. However, under temperatures of 35±0.05°C, it only took 14h for the pH value to drop from 7.528±0.037 to 6.143±0.031. The pH values decreased much more rapidly with increased PMI at higher temperature when compared with the values in the lower temperatures, which indicated that ambient temperatures play a significant role in estimating the PMI.

Regression analysis was performed for data obtained at different temperatures and PMIs. The PMIs and various pH values were used as variables, where x represented PMI and pH values as dependent variable y. The optimal regression function results showed that the R^2 value of the cubic curve-fitting equation was higher ranging from 0.974 to 0.982, suggesting that the degree of fitting was optimal, which indicated that there was strong correlation between the pH values and the PMI. The data also showed that postmortem changes were a dynamic process influenced by ambient temperature. A 3D surface equation was developed in estimation of PMI under various temperature conditions. Statistical analysis and curve-fitting of the data yielded cubic polynomial regression equations and a surface equation at different temperatures. The relationship among pH, PMI, and ambient temperature can be described by a three-variable fifth-degree equation. Interpolation analysis was applied to develop a 3D surface equation in estimation of PMI under various temperature conditions. The surface equation represented a 3D sculptured surface, indicating the relationship among pH, PMI, and ambient temperature could be descripted by a three-variable fifth-degree equation.

In conclusion, this study provides another useful tool of using interpolation analysis to estimate PMI at various ambient temperatures. The mathematical description using interpolation function fitting seems to be more suitable to cope with the complexity of PMI estimation in consideration of ambient temperatures.

pH, Postmortem Interval, Interpolation Analysis

G140 Postmortem Eye Temperature Measurement: A New Method of Time of Death Estimation?

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After attending this presentation, attendees will be familiar with a process of body cooling after death. The knowledge of cooling rates of different body sites is of use estimating the Time of Death (TOD).

This presentation will impact the forensic science community by showing the possibility of estimation of the TOD, especially in the early postmortem period, by measuring the temperature in the eyes of human corpses.

Estimation of the TOD is an important issue for the forensic pathologist examining the body at the death scene. The TOD estimation methods actually used in practice, based on postmortem changes such as hypostasis, rigor mortis, rectal temperature, or different supra-vital reactions are still not of satisfactory precision.

Recent studies in pigs and humans have shown a possibility of significantly decreasing the TOD estimation error, in particular

regarding the very early postmortem period, by choosing the eye as the temperature measurement site.

The single eye, rectal, and ambient temperature measurements were taken at the scenes of death in 21 cases with known TOD (1h 35min to 5h 15min), using pin probes connected to a high-precision electronic thermometer (Dostmann-electronic). The measured eye temperatures ranged from 20.2°C to 33.1°C. Rectal temperatures were measured at the same time and ranged from 35.0°C to 37.4°C. Ambient temperatures (1°C to 24°C), environmental conditions (still air to light wind) and hair amount on the head were also recorded every time. TOD was calculated using a formula based on Newton's law of cooling previously successfully applied in comprehensive studies on pigs: $T = T_a + (T_0 - T_a)exp(—k_c t)$ (Eq. 1) where T is the temperature of the body site, T_a is the ambient temperature (assumed to be constant during the course of cooling until the time of the measurement), T_0 is the initial human eye temperature (assumed to be 34.9°C), k_c is a first order cooling rate constant, and t is the time since death. The mean value of k_c=-0.113h^{-1} had been previously determined in studies on the postmortem cooling process in pig eyeballs. Thanks to both the significantly faster postmortem decrease of eye temperature and the residual or lack of plateau effect in the eye, no influence of body mass, TOD in the human death cases using eq. 1 could be estimated with quite good accuracy. The maximum TOD estimation error during the postmortem intervals up to around 5h was 1h 28min in one case among 21, while for the rest of 20 cases it was not more than 53min, while mean error for all 21 cases was ± 49 min. However, this highest overestimating TOD error in one case may be explained by baldness of this one examined individual.

The results from 21 cases with known TOD show that the presented method of TOD estimation may be of satisfactory accuracy in the early postmortem period, particularly when applied to bodies found at room temperature and in normal environmental conditions (still air, normal humidity). The study is being continued and the model is going to be improved using the new results from successive cases.

Time of Death, Body Cooling, Eye Temperature

G141 Toxicology of the Exhumed Body: Challenges and Pitfalls

Enrico A. Risso, MD, Via Alfredo Catalani 10/26, Genoa, ITALY*

After attending this presentation, attendees will improve their understanding about the "nightmare" of postmortem drug redistribution.

This presentation will impact the forensic science community by providing results from several studies in a topic which is often underestimated in the textbooks. This presentation is a proposal of guidelines for toxicology sampling in case of exhumation, including the most important sampling steps which have to be performed at the locus.

The word exhumation derives from the Latin words *ex* and *humus*, the former meaning "out of" and the latter meaning "ground." These words mean "out of ground" and describe the legal and authorized procedure to retrieve the coffin and the body from a grave for postmortem examination after legitimate burial in a cemetery. This event represents a real crime scene and has to be approached by pathologists and police officers as such.

Although autopsy procedures on corpses buried for a long time and in an advanced state of decomposition were regarded to be useless, without being accepted by the scientific community until the end of the nineteenth century, recent studies have proved that many useful histological and toxicological findings can be identified after months or years of interment. In several countries exhumations are extremely rare. Therefore, even for the most experienced forensic pathologist, it might be very difficult to develop the appropriate knowledge and skills to approach a forensic exhumation with the particular interpretative problems that might arise during the analysis of the findings.

The most famous exhumations performed in the United Kingdom (U.K.) were the victims of Dr. Harold Shipman, an English General Practitioner who shocked the population of his nation by killing up to an estimated 220 – 240 of his patients with lethal doses of diamorphine. Recent famous exhumations were performed in Italy in 2007, when the bodies of Poliziano (1454 – 1494), a Florentine classical scholar and poet, and Pico della Mirandola (1463 – 1494), a Renaissance philosopher from Modena, were exhumed from St. Mark's Basilica in Florence. Toxicological analyses established that both had died as a result of arsenic poisoning. Another extremely interesting item of research was recently performed on Napoleon's hair in France. The toxicology result undermined the theory that the British poisoned him with arsenic and disclosed that the pattern of arsenic consumption was spread over a long period of time, reflecting a chronic exposure rather than an acute poisoning; therefore, if the cause of death was due to arsenic poisoning, the theory of conspiracy which implicates one of Napoleon's companions poisoning him only while on St Helena's island would not be possible.

Although it is well known that arsenic and other heavy metals (such as antimony, zinc, copper, lead, mercury, cadmium, and thallium) may normally be present in the soil, a higher concentration within the human tissue clearly excludes the possibility of passive diffusion from the earth into the corpse. Mercury can persist in bones for thousands of years and it has been isolated from prehistoric human remains. High aluminium levels in bone, brain, and liver are regarded to be the most likely cause of death in exhumed bodies of dialyzed patients.

Postmortem toxicology in case of exhumation, especially after several years of burial, is indeed a very challenging matter and, as mentioned above, a failure during the sampling may result in a wrong interpretation of the data even for an experienced forensic pathologist.

Toxicology, Exhumation, Sampling

G142 Body Packages in Spectral Imaging CT-Experimental Study to Distinguish Between Different Illicit Drugs *In Vitro* and in a Porcine Model

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After attending this presentation, attendees will understand the impact of modern imaging methods on research and clinical handling of suspected body packers as well as the need for cooperation between forensic and clinical disciplines.

This presentation will impact the forensic science community by demonstrating how new imaging methods like dual energy spectral imaging CT scan alter existing algorithms and change clinical handling of suspects and patients, provided that specialists from the disciplines involved cooperate closely.

Bodypacking—meaning the transport of packed illicit drugs inside the human body—is a prevalent and increasing concern for modern societies with a major impact on the medicolegal and clinical field. The most feared complication of body packing is the rupture of one of the carried packs with potentially lethal drug intoxication (body packer syndrome). When suffering from symptoms of intoxication due to pack rupture, existing algorithms for the treatment of body packers suggest immediate abdominal surgery to remove the packs. Yet, in cases of heroin intoxication, a laparotomy with its high rate of complications could be avoided by administration of the antidote

Naloxone and intensive care supervision of the patient. Thus, the distinction of carried illicit drugs, especially between heroin and cocaine, can be crucial in clinical handling of body packers and influence medicolegal investigations. Our objective was to evaluate the differentiability between several substances in packages containing illicit drugs via Spectral Imaging CT (SICT) *in vitro* and in a porcine model.

Ten samples of illicit drugs (heroin, cocaine and hashish in different concentrations/compressions) packed in standardized ovoid plastic containers were examined *in vitro* and after placement in the rectum of a 121.5kg pig cadaver. Images were obtained using a 64-row CT unit (GE CT750 HD) in spectral imaging mode. The mean CT number in Hounsfield-Units (HU) representing radiation density was recorded for each sample. Spectral curves displaying radiation density of each sample in HU in relation to photon energy at keV-levels between 40 and 140 were obtained. Mean HU and standard deviation as well as the average slope (S) of the curve were evaluated for each sample. Statistical analysis was performed using Wilcoxon's test.

Mean HU of a substance was greatly affected by its concentration and the degree of compression and did not significantly differ between the investigated drug types. *In vitro*, the average slope of a substance's spectral curve did not significantly differ with varying density and concentration, but was characteristic for the investigated type of drug (i.e., heroin, cocaine, and hashish, respectively; all $p<0.001$). In the porcine model, differences were less pronounced than *in vitro* but still significant ($p<0.01$). Mean effective radiation dose for the scans was 9.4 mSv in the porcine model.

This presentation will demonstrate that interdisciplinary cooperation is important to evaluate potential applications and use the advantages of new imaging methods to their full extent. It will highlight the ability of new imaging methods to differentiate between various substances in general and different illicit drugs in particular. In contrast to hypotheses in the existing literature, the examinations *in vitro* and in a porcine model suggest that different illicit drugs cannot be identified by measuring their radiation density in HU at any given keV level. However, at a reasonable radiation dose, SICT may aid in the identification of incorporated substances in a body packer using the slope of the spectral HU curve. If confirmed in clinical studies, this information could alter the clinical handling of symptomatic body packers suffering from body packer syndrome due to pack rupture. Body packers carrying heroin could be treated conservatively instead of undergoing complicated abdominal surgery.

Body Packer, Illicit Drugs, Spectral Imaging CT

G143 Death Following Retrobulbar Injection of Desmopressin for the Treatment of Non-Arteritic Anterior Ischemic Optic Neuropathy: Which Implications of an Off-Label Use

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After attending this presentation, attendees will be aware of the possible clinical and legal implications following off-label use of corticosteroids and desmopressin for the treatment of Non-Arteritic Ischemic Optic Neuropathy (NAION).

This presentation will impact the forensic science community by illustrating a case of fatal acute myocardial infarction after retrobulbar injection of synthetic replacement of vasopressin.

Since a standard treatment for NAION with proven efficacy is not available to date, most therapeutic approaches are empirical and include a wide range of agents presumed to act on thrombosis, on the blood vessels, on the disk edema, or presumed to have a neuroprotective effect. Among other proposed treatments retrobulbar injection of corticosteroid and desmopressin represents an invasive approach, with potential local and systemic complications. Which is believed to have never been documented before. The rationale for using desmopressin in the treatment of NAION is uncertain and probably consists in experimental findings demonstrating that desmopressin induces ciliary artery relaxation in dogs via V1-receptors through a mechanism which involves nitric oxide. In turn, this would enhance vascular permeability, thus facilitating the re-absorption of optic edema. A similar vasodilation effect can be attributed to corticosteroids.

A 60-year-old man, apparently healthy with negative history for cardiovascular diseases, was hospitalized because of a unilateral sudden and painless severe visual loss (20/200) on waking in the morning. The optic disc appeared hyperemic and edematous, with a focal severe swelling. Relative inferior altitudinal scotoma was present at visual field examination. The patient presented with erythrocyte sedimentation rate 20mm/h and normal levels of plasma fibrinogen and C-reactive protein. The diagnosis of NAION was made. Two separate and immediately consecutive injections of betamethasone (2mg/0.5ml) and desmopressin (2mcg/0.5ml) were performed in the retrobulbar space. The administration of any preoperative medication or cardiovascular examination is not documented. In the patient medical records, the total volume of the injections and the size of the needle used are not specified. The procedure was technically uncomplicated without any monitoring in progress during injections such as ECG, peripheral oxygen saturation, or blood pressure measurement. Fifteen minutes later, the patient suddenly developed cold sweat, dyspnea, thoracic pain and severe hypotension. ST segment elevation Acute Myocardial Infarction (AMI) was diagnosed by ECG. Intensive care support was initiated. However, despite cardiopulmonary resuscitation, the patient died of irreversible cardio-respiratory arrest. At autopsy, the heart presented with a normal shape and weight (350g); coronary arteries showed significant atherosclerotic luminal narrowing. Histological investigation showed a stenotic atherosclerotic plaque (95%) complicated by culprit thrombosis of the Left Anterior Descending (LAD) artery, 2cm after its origin. Examination of the other organs was unremarkable, except for mild pulmonary edema and polyvisceral stasis. There was no evidence of increased orbital volume or sign of vagal compression secondary to retrobulbar hemorrhage.

Professional autonomy in the health care decision-making process renders the physician free to prescribe a drug for purposes other than that which it has been approved, where it is considered both safe and effective according to his/her professional judgement.

The use of unlicensed and off-label medicines is a widely used medical practice mainly in certain clinical settings.

Physician's autonomy in healthcare decision making represents an instrument to guarantee the progress and evolution of scientific knowledge and to realize in the meantime the most effective safeguard of health protection and promotion.

Though off-label prescription is common and sometimes necessary for providing a pathway to clinical practice innovation, it presents significant risks. This practice may lack rigorous scientific scrutiny and there is little known about the degree of scientific evidence supporting it. According to the literature, a high percentage, around 73%, of off-label use had little or no scientific confirmation.

Unexpected, adverse effects represent a possible risk. Since the off-label practice may expose patients to avoidable risks, it is mandatory for doctors to follow the lawful direction and ethical recommendation.

This case is out of the ordinary for the forensic scientist because of the possible recognition of medical liability for personal injuries and murder when a patient has psycho-physical damage or dies as consequence of the administration of an off-label drug.

Desmopressin, Non-Arteritic Anterior, Off-Label Prescriptions

G144 A Case of Suicide by Nembutal: The Consequences of Free Trafficking Drug Online

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The goal of this presentation is to show a suicidal case of a veterinarian and/or human barbiturate euthanasia agent (Nembutal), purchased freely online. Postmortem findings with a complete histological and toxicological analysis of pentobarbital distribution in fluids and tissues are discussed.

This presentation will impact the forensic science community by showing the importance of an accurate anamnestic, circumstantial, histological, and toxicological investigation in all drug-related deaths. At the same time pointing out that pentobarbital is not available in pharmacies as a commercial preparation, but its online traffic is completely free for anyone.

Pentobarbital is a hypnotic belonging to the class of rapidly acting barbiturates. Sodium pentobarbital, which is also called "truth serum", was adjunct during narcoanalysis to allow increased interaction of catatonic and schizophrenic patients with therapists, particularly during the treatment of these patients during the 1930s and 1940s. Today it is commonly used in pharmaceutical preparations for the euthanasia of animals. On the one hand, accidental and volunteer overdose caused by short-acting barbitures in humans have become rare since these molecules became commercially unavailable as sleeping drugs/hypnotics. On the other hand, suicidal ingestion of a lethal dose of pentobarbital has increased because it is advocated by the "Exit" association. This association provides assistance to individuals wishing to commit suicide because of lasting painful disease or severe degradation of their physical condition. Even if the pentobarbital is not available in pharmacies as commercial preparations, it could be ordered with a medical prescription from local drug distributors; its online trafficking is completely free, as this case demonstrates.

A 24-year-old male was found dead in a bedroom of a bed and breakfast by an attendant. He was a fourth-year medical student who had supported a few tests, when, two years after the end of a relationship, he fell into depression. Followed by specialists, a "bipolar disorder" was diagnosed. In February 2012, his mother was aware that the young man had bought online from Mexico a drug used in veterinary medicine: Nembutal. Obtaining information about the shipment, the woman was able to seize the substance. Twenty days before his death, the mother had been informed by a friend about another online purchase of Nembutal from China. It arrived by mail but the parents were unable to seize the substance. On April 3, 2012, the young man pretending to sleep at a friends, reserved a room at a bed and breakfast, where he was found dead the following day at 1 p.m. The corpse was laying on the bed; and, next to him, an empty bottle of whisky and two packets of white dust (one empty and one full) were found.

A complete autopsy was performed 24 hours after death. The external examination revealed traumatic lesion. The internal examination revealed only polivisceral stasis and massive pulmonary and cerebral edema. Histological examinations showed, in the brain, cytotoxic and vasogenic edema and red neurons; in the myocardium, interstitial edema; in the lungs, massive endoalveolar edema; in the liver, sinusoidal stasis and macrovesicular steatosis. A comprehensive toxicological screening was performed on postmortem cardiac blood, urine, bile, gastric contents, and tissue homogenates (liver and kidney) using a combination of immunoassay and chromatographic techniques. In all the biological fluids and tissues, lethal concentrations of pentobarbital were confirmed (blood: 57,461mcg/mL; bile: 890,67mcg/mL; urine: 7.06mcg/mL; gastric contents: 13474,96mcg/mL; liver: 512,48mcg/mL, kidney: 361,77mcg/mL). Ethanol and volatile compounds in peripheral blood were sought by head-space-gas-chromatography equipped with flame iosination detection (HS-GC-FID); it showed a very high level of alcohol in the blood (1,2903g/l). The presence of pentobarbital was also confirmed by toxicological examination of the white dust.

According to the crime scene data, autopsy, histological, and toxicological findings death was attributed to acute pentobarbital intoxication.

Pentobarbital, Drug Online Trafficking, Toxicological Finding

G145 Income-Based Medicine and the Avastin Dilemma: How Italian Doctors Carry the Tangled Skein on Their Shoulders

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After attending this presentation, attendees will learn about distributive justice concerns and their consequences on medical liability.

This presentation will impact the forensic science community by illustrating how evidence is not a value in itself, but needs to be interwoven with other societal values in a clear way.

The treatment of age-related macular degeneration has opened a wide-ranging discussion. Although ranibizumab (Lucentis) is a licensed and approved treatment, many ophthalmologists in Italy and worldwide continue to prescribe bevacizumab (Avastin), which is licensed for treatment of some metastatic cancers but not for the treatment of eye conditions. The off-label use of bevacizumab appears to produce comparable results, as shown by guidances of scientific societies worldwide and by the CATT Research Group, at a substantially lower cost than the licensed treatment of ranibizumab.

The practice of medicine is no doubt based on the appraisal of scientific evidence and, in such a sense, the drug licensing process plays a fundamental role. Nonetheless, the practice of medicine has long since shown universalistic attitudes.

The debate Lucentis-Aventis unveils the controversial dispute between the mere devotion to scientific evidence in clinical practice and the relative weights that some other elements (e.g., sensitive and cost-effectiveness analysis) play in medical decision-making. In the first hypothesis, the off-label use of drugs would be discouraged in favor of licensed drugs. This approach would have the side-effect of dramatically restricting the universalistic access to healthcare. In the second hypothesis, criticisms are grounded on the difficulty of balancing the role that empathy, cost-effectiveness, or other criteria should play in distributive justice. Given the need to prove the equivalence of therapeutic effect, pharmaceutical companies have not always demonstrated responsiveness to social and economic context, due to the profit loss subsequent to more cost-effective treatments. In addition, in the absence of clear guidances for the unlicensed or off-label use of drugs by regulatory authorities, it would be legitimate to doubt the universalistic attitude that inspires such controversial medical practice, as universalism is patently bounded to efficacy.

In both hypotheses, there is a small risk to subsume scientific evidence to profit maximization (income-*based medicine*), given that collection and appraisal of evidence risk to be conditioned by actual and prospective earnings of pharmaceutical companies without any referral to positive consequences for the health status of the society. In such a context, given that the responsability for the off-label use of drugs rests on the shoulders of prescribing doctors, the decision between the aforementioned hypotheses appears to be dependent on the individual professional person, directly bringing about unfair

inequalities. In addition, the legal protection for those clinicians who act cost-effectively appears to be lacking under the Italian laws, on the basis that incomplete scientific evidence could be given to prove one's diligence in case of off-label use of drugs. The controversy is relevant, because the relatively wide scientific evidence gathered in favor of the off-label use of bevacizumab is uncomparable with that available in other clinical settings (e.g., Neonatal Intensive Care Units), where the off-label use of drugs is nonetheless common, even though the information about the optimal dosage, specific pharmacokinetics characteristics, as well as potential adverse reactions, is insufficient.

Off-Label Drugs, Medical Liability, Ethics

G146 Fatal Sodium Nitrite Poisoning: A Case Report

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After attending this presentation, attendees will learn about acute intoxication deaths resulting from unusual chemicals, representing a challenge for investigators and pathologists in charge of determining cause of death.

This presentation will impact the forensic science community by showing that only a collaborative and multidisciplinary approach involving public health officials, forensic pathologists, and toxicologists can properly lead a comprehensive investigation.

Sodium nitrite poisoning, usually due to accidental food and water contamination, is rarely recorded in the literature. Sodium nitrite is a white to slightly yellowish crystalline powder that is very soluble in water. Its main use is for the industrial production of organo-nitrogen compounds, but it is also used as a food additive because it inhibits growth of pathogenic microorganisms and is used as a taste and color preservative for certain meats. It can be toxic in high amounts for humans because it causes methemoglobinemia. Sodium nitrite's LDLo is 33-250mg/Kg, meaning a 60kg person would likely have to consume at least 2g to 15g to result in death.

Presented is a case of "iatrogenic" sodium nitrite poisoning that occurred during a medical examination and malabsorption test for three patients, resulting in the death of one of them. Three women performed a Sorbitol H2 Breath Test to detect specific sugar malabsorption. Acute-onset symptoms, consistent with chemical poisoning, occurred within minutes after consuming 5g of sorbitol dissolved in a glass of water. Symptoms included asthenia, lost of consciousness, myoclonic jerks, nausea, and retching. The emergency was immediately recognized, but unfortunately, one of the women died a few minutes later despite all efforts and administration of methylene blue in an attempt to revert methemoglobin to hemoglobin. The other two women were transported to the nearest emergency department where methylene blue was promptly administered. The two patients were later discharged without clinical sequelae after hospitalization in the Intensive Care Unit. Samples of the presumed sorbitol powder that had been administered to the women were collected by authorities in the private medical clinic where the breath test had been performed. Investigations revealed that sorbitol had been bought online (international website) and that it was the first time it had been used in this clinic. During the autopsy, the external examination of the cadaver revealed diffuse cyanosis and resuscitation signs. Postmortem findings (organ gross examination and histological analysis) confirmed a normal cardio-respiratory system apart from the evidence of multiorgan congestion and pulmonary intra-alveolar hemorrhagic edema. The gastrointestinal apparatus showed acute congestive and focal hemorrhagic findings of the visceral wall related to the contact with poison. The majority of organs also showed a bluish discoloration due to the intravenous infusion of methylene blue as a result of the attempt at resuscitation. Extensive toxicology testing was performed to determine the cause of the poisoning and a list of potential agents was developed by forensic toxicologists. Infrared spectroscopy and gas chromatography revealed that all the sorbitol recovered in the clinic was actually sodium nitrite at a concentration of 97 – 98%. Analyses of urine and blood collected at the time of autopsy were performed using gas chromatography and test for nitrites and nitrates; the presence of high concentrations of sodium nitrite was confirmed. The serum nitrite ion level was 0.97mcg/ml and this level is consistent with death from nitrite poisoning (lethal level >0.55mcg/ml). The small concentration of nitrite in urine suggested the occurrence of rapid death.

The health risk of purchasing drugs from online websites that are not official drug resellers of well-known companies will be debated.

Sodium Nitrite, Sorbitol Breath Test, Toxicity

G147 Postmortem Toxicological Review of Combined Drugs Toxicity Deaths Reported in the Republic of Ireland Involving the Detection of Bath Salts Headshop Products

Khalid Jaber, MD, Firebrigade Training Centre, Malahide Rd, Dublin, IRELAND*

The goal of this presentation is to share the Irish experience in identifying deaths caused by combined drug toxicity in which detection of headshop products, referred to as bath salts, has been identified.

This presentation will impact the forensic science community by sharing, for the first time, the Irish experience of chemical deaths caused by combined drugs toxicity that postmortem toxicoloigcal analysis detected as headshop products (drugs), referred to as bath salts. The human toxicology section of the state laboratory is a centalized toxicological analysis unit that handles all referred samples from the various coronial services operating in the Republic of Ireland.

Synthetic cathinones include drugs like mephedrone, MDPV, butylone, flephedrone, and methylone that form part of a larger group of illegal drugs encountered in Ireland and other countries as components of products sold in retail stores referred to as head shops.

In Ireland, prior to May/June 2011, a rapid growth of these commercial interests was noticed on the main streets in the capital of Dublin and in most other smaller cities and towns in Ireland. The public as well as the media have become alarmed with the proliferation of the head shop products. A national TV documentary program focusing on a downtown Dublin street recorded 18 customers that queued, over an interval of 15 minutes of filming, in front of a small hatch of one head shop. They were buying these products for cash. Ireland was experiencing a new epidemic of addiction craze of consumption of head shop products, which have been marketed as bath salts although marked "not for human consumption," with 24 hr availability and facility for home delivery to those requesting such service. These products for some time have been legally cleared by customs when imported by these business outlets. An owner who was interviewed articulated his position that he was selling bath salts and it was up to the buyers to use them the way they saw fit. For example, one gram of his bath salts could sell from 10 to 30 euros!

The consumers of these products (the customers of these shops) spanned a wide range of ages (technically, customers must be over 18 to buy anything from a head shop) and represented all social strata, both sexes, and marital status.

Users' clinical signs and symptoms such as hallucinations, anxiety panic attacks, and psychosis were increasingly observed and reported to health workers; the police force was monitoring the rise of this worrying trend. These drugs have become a convenient replacement to the hard drugs of abuse as consumers began to inject these drugs. In November 2009, the first fatality of combined drug toxicity that included mephedrone was strongly suspected initially and was subsequently confirmed in postmortem toxicological analysis. In

February 2010, the Department of Health placed certain substances on sale in head shops under the control of the Misuse of Drugs Act 1977, paving the way for these bath salts products to be outlawed. In the same month, an arson fire totally destroyed a head shop in Dublin. In March 2010, two pipe bombs were discovered by employees of two head shops separately. The Army Bomb Disposal Unit removed the weapons and deemed them to have been two viable improvised explosive devices. The following month, a petrol bomb attack was carried out on a head shop in a seaside town north of Dublin, leading to fire breaking out.

In May 2010, the Irish government announced a criminal ban with immediate effect on a list of head shop-based drugs, making it illegal to trade in mephedrone, spice products, and substances that exert biological effect like cannabis, cocaine, and ecstasy.

One month later, the Minister for Justice published the New Criminal Law, The Psychoactive Substances Bill, which made it illegal to sell hallucinogenic products. The bill granted power to Garda Siochana to seek a court order to close head shops suspected of selling drug-like products; the owners of these premises were required to prove they are not involved in such activities.

The state laboratory identified 33 postmortem toxicological analyses that showed positive head shop synthetic cathinones over a three year period between November 1, 2009 and November 30, 2011. Five cases were identified prior to the New Psychoactive Substances Bill while four cases were identified in June 2010 and the rest afterward. These cases comprise twenty-six males and six females.

An account of these cases, discussion of this epidemic, and the effectiveness of the national measures taken is presented.

Toxicology, Headshop, Drugs

G148 Cause of Death–Acute Alcohol Poisoning, Manner of Death–Suicide: A Case Study

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After attending this presentation, attendees will understand the importance of alternative or secondary specimens collected at autopsy for the toxicological analyses of volatile compounds, most notably, ethyl alcohol or ethanol. The case data represents exceptionally high alcohol concentrations in heart blood and vitreous humor within the context of the death scene investigation and autopsy findings. Interpretive considerations when evaluating unusually high blood and vitreous humor alcohol concentrations, with special attention directed to possible explanations for the findings based on the decedent's history and scene investigation, are included in the case study.

This presentation will impact the forensic science community by showing the significance and importance of a complete, competent medicolegal death investigation integrated within a diligent scene investigation, an autopsy with appropriate collection of primary and alternative specimens for toxicological study, and most importantly, the collaborative work effort of medical death investigators, forensic pathologists, and forensic toxicologists.

Acute alcohol poisoning is a frequent cause of death classification; however, it is rare when a decedent willingly and intentionally initiates a heavy bout of drinking with the sole purpose of consummating a life-ending act. The case is an 85-year-old male found deceased, sitting upright in a chair, in a ground floor room of his private residence. The witness, who reported the death, stated the decedent was sitting in the same manner two days before; the relative believed the man to be asleep and did not want to disturb him. The witness confirmed the decedent alive three days before but apparently in a state of distress; he also appeared to have been intoxicated and crying. His family history was notable for the death of his spouse approximately six weeks earlier. Investigation at the scene revealed a hand-written note simply stating "good bye." Further, two books found in the residence (*Final Exit* and *The Peaceful Pill Handbook*) extolling the practicalities of "self-deliverance" strongly suggested ideation on the part of the decedent. Additional evidence collected from the scene included a store receipt for a 1.75 liter bottle of vodka with a purchase date of three days prior to discovery of the body. Investigators discovered an empty vodka bottle under a bed in an upstairs room. External examination of the body indicated rigor, lividity, and skin slippage to be consistent with a postmortem interval of three days. Remarkable findings at autopsy included severe plaquing of the coronary arteries and aorta, a partially calcified aortic valve, moderate plaquing in the cerebral arteries within the Circle of Willis, notable cystic changes in the kidneys, and pulmonary edema. Autopsy noted moderate decomposition of the body along with an absence of significant injury capable of causing or contributing to death. Specimens for toxicological analyses collected at autopsy included heart blood and vitreous humour. The analyses consisted of volatile compounds by headspace gas chromatography in blood and vitreous humour and a comprehensive screen (more than 300 compounds) in blood by Ultra Performance Liquid Chromatography coupled to Time of Flight Mass Spectrometry (UPLC/ToF-MS). The results of the volatiles analyses indicated alcohol concentrations (weight by volume) of 1.605% and 0.893% in blood and vitreous humour, respectively.

Previous studies in cases involving unusually high alcohol concentrations demonstrated a mean blood alcohol concentration of 0.74%, with a range 0.42 – 1.77%. A report also indicates a 55-year-old male achieving a blood alcohol concentration of 1.13% (w/v) in a suicide gesture averted by supportive therapy.[1] This case study explores the plausibility of achieving the alcohol concentrations reported for heart blood and vitreous humor and includes discussion of other factors (diffusion, postmortem neo-formation secondary to decomposition) to consider when interpreting the results.

Reference:

1. *In:* Disposition of Toxic Drugs and Chemicals in Man, 9th ed., Randall C. Baselt, Biomedical Publications, Seal Beach, California, 2011

Ethanol, Poisoning, Suicide

G149 Decomposition Pattern of Human Heads as Related to Insect Activity

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After attending this presentation, attendees will understand how human heads decompose with the involvement of insects and the different way in which insect activity proceeds depending on whether a head is covered or uncovered.

This presentation will impact the forensic science community by studying the process by which human heads decompose through the activity of insects.

Despite the important role of insect activity in the process of human decomposition, decomposition pattern by insect activity has been an issue of little interest among forensic anthropologists as well as forensic entomologists. The goal of this study is to examine and compare the decomposition patterns of human heads in covered and uncovered conditions through the evidence of insect activity. Heads, as opposed to full cadavers, were chosen for this study because of their forensic significance. Blow flies tend to lay eggs in the head region first, and according to an unpublished preliminary study, the head revealed the most consistent decomposition pattern compared to other body parts.

This research was conducted at the Anthropology Research Facility at the University of Tennessee, Knoxville. A total of 60 donated

individuals (36 covered and 24 uncovered), donated between April 2011 and March 2012, were observed and photographed every day until no further taphonomic change was observed.

The results of this research indicate that covered heads were more likely to reach skeletonization than uncovered ones. Specifically, while about 78% (28 out of 36) of covered heads exposed more than half of cranial bones, only 25% (six out of 24) of uncovered heads reached the same degree of decomposition. This result is statistically significant (χ^2=16.33, p=.000). For both covered and uncovered heads, newly-hatched maggots tend to start feeding inside the orifices, among which the mouth and nose are most popular. Observations show that as the maggots grow, they move out of the holes, exposing bones around the T-zone of the face which connects eyes, nose, and mouth.

Further study observations demonstrate that when a head is covered by black plastic sheeting, maggots coming out from the mouth, nose, and eyes tend to congregate on relatively high portions of the head (i.e., the surface furthest from the ground). While some maggots travel on the outer skin, many pass through the narrow space between bones and inner skin, exiting through holes that they make. After conquering the high portion of the head, maggots consume soft tissues downwardly, resulting in the exposure of bones far from the ground first. Thus, for covered heads, any remaining, soft tissues closest to the ground are more likely to remain and dry out relative to tissues further from the ground.

Conversely, observations from the study show that when a head is not covered, maggots coming out from the holes tend to gather together toward the ground, which usually is a shaded area. Because they tend not to feed on the area directly under the sun, soft tissues facing the sky are likely to remain untouched and dry out. Thus, for uncovered heads, any remaining soft tissues close to the ground are more likely to be skeletonized, demonstrating an opposite pattern to that of the covered heads.

In summary, this research suggests that black plastic sheeting functions to block sunlight, which in turn provides maggots with a relatively comfortable environment to feed. Consequently, this particular environment significantly alters the decomposition pattern of heads. The information of this study is expected to be utilized during crime scene investigation when it is necessary to determine if a body was covered during the initial period of its decomposition and if the body was disturbed after its deposition.

Decomposition, Pattern, Insect Activity

G150 PMI Estimation in Burned Remains: Real Cases

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After attending this presentation, attendees will have novel information about the applicability of the Forensic Entomology (FE) approach for the Minimum Time of Death (mPMI) estimation in burned remains.

This presentation will impact the forensic science community by validating the entomological approach in the PMI estimation in burned bodies. The two reported cases, and the experiments carried in the same area, statistically confirm the applicability of the entomological approach based on the fly developmental rate for the PMI estimation.

Different methods can be used in order to destroy or hide a body or make its identification very difficult: mutilation, burning, burying, acid dissolution, and concealment into concrete blocks, among others. Burying and burning are perhaps the most common ones. Fire is also used in cases of self-inflicted injuries and suicide or suicide-homicide, but burned remains can also be found in several accidents of different origin (cars, aircraft crash, industries, domestic, etc.), and cases of burnt bodies found in different locations such as open fields, cars, and indoors have been reported by several authors.

In burnt bodies, arthropod specimens may be the only tool useful in the estimation of the mPMI. In fact, burning prevents the use of the classical thanato-chronological techniques (e.g.: livor, rigor, algor mortis, (K^+)) for mPMI estimation.

This work deals with the description of five burned bodies found during the summer 2011, in Central Italy. The studied cases can be grouped in two categories: homicide/suicide (three people) and aircraft crash (two people).

In July 2011, a mother killed herself, her daughter, and her son by administering a drug and setting fire, using gasoline, to the car in which they were located. The last time they had been seen alive was 3:08 p.m. Fire brigades were alerted at 3:38 p.m. When forensic pathologists arrived on the scene, the bodies were completely burned and colonized by fly eggs. Most of the colonization in the mother's body was at the abdomen and inside the skull, fractured by the heat. During the autopsy, performed the day after, the larval mass in the mother's abdomen had a temperature of 35°C, despite the bodies being stored in a refrigerator.

Colonization in the daughter's body was less important than in the other bodies. There was only a mass in the skull and the larval mass temperature was 5°C. In the body of the son, a mass in the chest (temperature was 19°C) and another mass in the abdomen (24°C) were observed. *Lucilia sericata* and Sarcophaga sp. larvae were collected from these masses. It is assumed that the fly oviposition and larviposition occurred immediately after the fire had been extinguished and before the bodies were recovered (two to four hours).

An air crash occured at 7:07 p.m. in July 2011. During the inspection at 8:00 p.m., the air temperature was 25°C. On the ground near the fuselage of the aircraft, firemen collected the broken airplane clock indicating seven hours, seven minutes, and 40 seconds, so the precise time of the impact and of the death (7:07 p.m. 40 seconds) was known. Two people died during the crash. The first body was only partially charred and did not present any fly colonization; the second one was almost completely charred and did present a colonization (eggs) in the lungs, heart, and liver. The fly oviposition (*Lucilia sericata*) occurred immediately after the fire had been extinguished and before the bodies were recovered (two to four hours)

These cases demonstrate that, in burned remains, the composition and the arrivals of the first insect colonization waves (Calliphoridae, Muscidae, Sarcophagidae) are the same as in the case of "fresh" bodies. In addition, the aircraft crash case indicates a faster colonization of the burned body compare to the other. This supports the application of the entomological approach, based on the developmental rate, for PMI estimation.

PMI Estimation, Forensic Entomology, Fire

G151 Initial Investigations of Hyperspectral Remote Sensing for Postmortem Interval Estimations in Forensic Entomology

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After attending this presentation, attendees will be introduced to the initial applications of hyperspectral remote sensing to forensic entomology and understand its potential for increasing the precision of current immature blow fly age estimates and successively increasing the accuracy of postmortem interval estimates.

This presentation will impact the forensic science community by illustrating the opportunities offered by hyperspectral remote sensing into an entirely new area, entomology, and its possible future potential value in forensic or medicolegal entomology.

Current methods examining dipteran larval development to estimate minimum elapsed time since death are based on a minimum tenure of the insects on the body. Immature blow flies develop at a predictable rate and, based on the insect stage, a minimum postmortem interval is estimated. Unfortunately, in some instances, because some of the stages can be lengthy, only a crude estimate can be provided. This initial proof of concept investigation examines the use of hyperspectral remote sensing to reduce the estimate in the postmortem interval. The use of hyperspectral remote sensing in forensic entomology is unheard of, but remote sensing is successfully being applied to many other sciences including many forensic sciences. The objective of this investigation was to examine the use of hyperspectral remote sensing in increasing the precision of current blow fly (Diptera: Calliphoridae) age estimates and consequently increasing the accuracy of postmortem interval estimates

Daily spectral measurements of immature *Protophormia terraenovae* (R-D), a common blow fly, spanned the 325 – 1025nm range but the measurements were condensed to reduce excessive noise. Measurements were obtained using a handheld spectrometer once daily. Immature development was examined in two parts due to time constraints. Measurements were obtained from second instar to the pupal stage and from the beginning of the pupal stage until adult emergence. The adult colony was raised on milk powder, sugar, and water ad libitum. A CMP 4030 Conviron chamber was used to maintain the adult colony as well as the experimental animals. Development occurred at 25°C, a 14:10 (L:D) photoperiod of 20µmol of light and a relative humidity of 75%. The immature insects were raised on milk-fed veal liver in the Phytotron at McGill University, and then insects were transported daily to the spectrometer for measurement. Despite the transport, the average temperature recorded by a data logger remained at 24.6°C. Matlab R2011b and PRtools 4.1 were used to complete the spectral analysis. Also, a fourth order polynomial Satvitsky-Golay smoothing filter was applied. A forward feature selection was used to designate the top 25 discriminating bands.

This proof of concept investigation introduces a comparison of how the spectral signatures of *P. terraenovae* change from day to day within the immature stages. The results are more than promising and show a potential method for narrowing the original estimates and offering a better overall estimate of insect age, and therefore, postmortem interval estimation.

Protophormia Terraen, Hyperspectral Remote, Forensic Entomology

G152 Radiocarbon Analysis of Fly Pupal Cases to Estimate Date of Death

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After attending this presentation, attendees will understand the potential value of radiocarbon analysis of fly pupal cases associated with recovered human remains to estimate the date of death.

This presentation will impact the forensic science community by demonstrating how radiocarbon analysis of fly pupal cases can be used to estimate the date of death of the associated human remains, offering a less invasive sample alternative which yields more accurate results.

Beginning in the 1950s, atmospheric testing of thermonuclear devices produced elevated levels of artificial radiocarbon in the atmosphere that subsequently entered the food chain and were incorporated into the tissues of living organisms, including humans. These levels reached a peak in 1963 and, subsequently, have declined following the cessation of atmospheric testing. Research and casework have demonstrated how analysis of the radiocarbon content of human tissues can be used to estimate the date of death when compared to the documented values of the "bomb curve." Human soft tissues are especially useful in this regard since their radiocarbon values are relatively close to the atmospheric levels in comparison with skeletal tissues. Of course, following decomposition and skeletonization, soft tissues may not be available for analysis. Since fly larvae associated with decomposing human remains feed on the human soft tissues, the radiocarbon values of those soft tissues would be expected to be incorporated into the tissues of the larvae, including the puparial cases. The durable puparial cases can be found associated with human remains long after the adult insect emerged. Radiocarbon analysis of recovered puparial cases thus produce values consistent with the human soft tissues and can be used to estimate date of death.

Five human skeletons with preserved fly puparial cases and known death dates were located in the Human Rights Division of the Servicio Médico Legal in Santiago, Chile. Documented death dates were 1973, 1974, 1979, 1986, and 1986. Samples of puparial cases from each were submitted for radiocarbon analysis to an ISO 17025 accredited radiocarbon laboratory. The following summarizes the results for each of the five samples, listing the conventional radiocarbon age expressed in Percent Modern (pMC), the actual death date of the associated individual, and the date of intersect of the radiocarbon value and the bomb curve (using values from both the northern and southern hemisphere).

1. 131.2 pMC; death date of 1979; intersect dates 1978 – 1979
2. 140.3 pMC; death date of 1974; intersect dates of 1973 – 1974
3. 144.7 pMC; death date of 1973; intersect dates of 1972.5 – 1973
4. 122.5 pMC; death date of 1986; intersect dates of 1983 – 1985
5. 122.0 pMC; death date of 1986; intersect dates of 1983.5 – 1985

As indicated in the information presented above, radiocarbon analysis produced values that intersected the bomb curve either on the date of death or within one year of it.

When working with fly puparial cases associated with unidentified human remains, context and/or radiocarbon analysis of carefully selected human tissues can clarify if the puparia values relate to the earlier ascending aspect of the bomb curve or the later descending aspect. During recovery of skeletonized human remains, preserved fly puparia should be collected since they potentially provide valuable information on date of death of the associated individual.

Radiocarbon, Puparia, Remains

G153 Microbial Communities and Necrophilous Insects Associated With Cadaver Decomposition Islands in Southeast Louisiana

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The goal of this presentation is to provide attendees with knowledge of the microbial diversity and forensically important insects related to the five stages of decomposition.

This presentation will impact the forensic science community by demonstrating the importance of understanding biological and ecological aspects of both forensically important insects and microorganisms associated throughout the decay process.

Microorganisms in topsoil and forensically important insects are both pivotal in the decomposition of vertebrate carrion. Decomposition of vertebrate tissues results from both internal and external factors including autolysis, putrefaction, and diagensis, as well as scavenging of tissues by insects, vertebrates, and microorganisms. In forensic biology, there is limited information

available regarding the microbial communities associated with the decay of large vertebrates above-ground (*i.e*., cadaver decomposition islands). Most studies regarding microbial diversity and community structure are associated with profiling soil microbial populations, clandestine graves, or small-scale laboratory studies. However, the majority of decomposing remains of forensic interest (humans and poached wildlife) are recovered above-ground and often placed directly onto vegetation, leaf litter, soil surface, etc. Furthermore, soil microbes could be of particular forensic importance in later stages of decay when insect abundances have declined. From an investigative and evidentiary point of view, the more information one has, the stronger the case. Thus, this research was designed to produce a comprehensive database of microbial diversity, community structure, and succession using adult swine carcasses (i.e., human representative and reference model for forensic entomology research).

This study includes four series of seasonal experiments conducted in a woodland habitat in Hammond, LA, during the winter and summer seasons during 2010 – 13. The primary goals of this research are twofold: (1) to establish a microbial diversity database and successional patterns of soil microbes associated with cadaver decomposition islands in southeastern Louisiana; and, (2) to correlate the microbial soil profiles with the observed stages of decay and faunal succession patterns of necrophilous insects. Each seasonal experiment consisted of three adult swine carcasses (~60 – 160kg) placed directly on the leaf litter/soil surface. Each seasonal study was conducted for 12 months with sampling events and protocols varying for insect and soil core collections throughout the five stages of decay. Entomological data were collected both manually and using pitfall traps. Each sampling event included collection of insects both manually and using pitfall traps as well as two soil samples (~12cc each) collected beneath each carcass using a soil-coring device. Soil cores consisted predominantly of aerobic microorganisms associated with decaying detritus and organic-rich topsoil of the forest floor. Topsoil cores were also collected per sampling event at a control site approximately 15 meters away from the carcasses.

Processing of the soil samples included the following: soil characterization (soil texture, pH, total organic carbon, and total organic nitrogen), microbial enumeration, DNA extraction and purification, PCR amplification, T-RFLP analysis, and sequencing analysis. The primary sarcosaprophagous fly species associated with the swine carrion during the winter 2011 study were two Calliphoridae species (*Calliphora vicina* (Robineau-Desvoidy) and *Phormia regina* (Meigen)) and one Muscidae (*Hydrotaea leucostoma* (Wiedemann)). Data generated from this study will produce successional patterns of insects and microbes, which could potentially elucidate estimations of time since death for large vertebrate carcasses above-ground. More importantly, microbial topsoil diversity data will likely be more informative during later stages of decay such as advanced decay and putrid/dry remains. Data from the winter 2011 study (February 5, 2011 to December 6, 2011) will be presented.

Decomposition, Insects, Microbes

G154 Effects of Ketamine on the Development of *Chrysomya Megacephala* (F.) (Diptera: *Calliphoridae*)

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After attending this presentation, attendees will gain the knowledge about how the presence of ketamine affects the development of larvae of necrophagous flies feeding on cadavers.

This presentation will impact the forensic science community by showing forensic entomologists and forensic pathologists the effects of ketamine, a drug commonly used as an anesthetic, on the development of larvae of the forensically important fly *Chrysomya megacephala* (Diptera: *Calliphoridae*). The understanding of the larval growth pattern of necrophagous flies feeding on cadavers in the presence of certain drugs could lead to a more reliable estimation of postmortem interval in drug-related deaths.

It is widely accepted that the presence of certain drugs or poisons can affect the development of larvae of necrophagous flies feeding on cadavers. These effects should be taken into consideration in estimation of the Postmortem Interval (PMI) of drug-related deaths. In recent years, it has been reported that many drugs and poisons within cadavers can affect the developmental duration of larvae or pupal stages of necrophagous flies, and then affect the accuracy of the PMI estimation. Ketamine, first synthesized in 1962, is an NMDA receptor antagonist and a derivative of Phencyclidine (PCP). Ketamine hydrochloride is used in intravenous anesthesia in clinical practice to replace PCP. Illicit drug abuse has risen to epidemic levels over the last decade in China because of the implementation of its open-door policies. Abuse of ketamine has recently gained popularity in China and an increased trend of deaths due to ketamine intoxication has been noted during the past decade in central China. It is not known if the effects of ketamine on the development of necrophagous flies have been reported.

This study investigated effects of ketamine, a drug commonly used as anesthetic, on the development of larvae of the forensically important fly *Chrysomya megacephala* (Diptera: *Calliphoridae*). Larvae of the *C. Megacephala* were divided equally into four groups and were reared on artificial diets containing different concentrations of ketamine: 0.25µg/g, 50µg/g and 100µg/g in an environmental chamber. Samples were collected every 12 hours. The body lengths, weights of each larva, and developmental durations of each stage were observed and measured. This study demonstrated that ketamine, low temperature, and their synergistic action significantly suppressed the larval growth of *C. megacephala* ($P<0.001$). The inhibiting effects of ketamine on the growth of larvae by length and weight were most significant before the larvae achieved their maximum length and weight when compared with a control group ($P<0.001$), especially in the 25µg/g ketamine-treated group at a temperature of 24°C. The time that the larvae achieved the maximum lengths and weights were significantly delayed in the ketamine-treated groups when compared with the control group ($P<0.05$), which resulted in prolonged duration of larval and prepupal stages while shortening the duration of pupal stages especially at low temperature. No significant differences were observed on the larval maximum length and weight between the control group and the ketamine-fed groups at the same temperature. No linear correlations were discovered between ketamine concentration and growth rate of larval body length and weight. In the deaths of suspected ketamine intoxication, the delayed growth effect should be taken into account when using regression against larval weight or length to estimate PMI.

In conclusion, ketamine can significantly delay larval development of *C. megacephala*. Knowledge of the effects of ketamine on larval development of *C. megacephala* can be useful in the estimation of PMI in suspected ketamine-related deaths. Further research is needed to observe the effects of ketamine on the development of more species of necrophagous flies as different species may have different responses to the same drug.

Forensic Entomology, Ketamine, Chrysomya Megacephala

G155 Heat Generation in Maggot Masses and Its Effect on Larval Development

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After attending this presentation, attendees will understand the relationship between the size of a maggot mass and the temperature of its microclimate as well as how this mass-generated heat influences larval development.

This presentation will impact the forensic science community by highlighting the importance of incorporating mass temperatures into PMI estimates that are based on larval development.

Observed to arrive at a body within minutes of death, blowflies (Diptera: *Calliphoridae*) are frequently used as a biological clock in criminal investigations, aiding in the estimation of the Postmortem Interval (PMI). In forensic entomology, PMI is estimated based on the temperature-dependent developmental rates of known blow fly species. By combining temperatures recorded at the death scene with the known rates of development for larvae reared in temperature-controlled environments, it is possible to age individuals and, hence, estimate time of death. However, few studies take into consideration the mass-generated heat produced by larvae co-existing in an aggregation. This study investigates the relationship between the number of blow fly larvae in a mass and the amount of heat generated, as well as identifying the minimum mass size and elapsed time before any differences in accrued temperature achieve significance. This research also highlights the potential impact mass-generated heat may have on larval development rates and, hence, any subsequent PMI estimates.

Various sized larval masses (50 – 2,500 larvae) composed solely of *Lucilia sericata* (*Phaenicia sericata* in North America) were reared in the laboratory on racks of lamb ribs at a constant ambient temperature of 22°C. Using data loggers and a thermal imaging camera, mass temperatures were monitored and recorded at five-minute intervals for the duration of the feeding stage of development. Data was analyzed using the statistical package R (version 2.12.1). Results showed a strong positive relationship between mass size and the amount of heat generated by the aggregation, with temperatures rising as masses increased in size. A minimum mass size of 1,200 individuals was required for the microclimate temperature to increase significantly above ambient ($p \leq 0.05$), with aggregations composed of 2,500 larvae producing temperatures that exceeded ambient by as much as 14°C. Comparing Accumulated Degree Hours (ADH) for different-sized masses at specific times during development highlighted 26 hours as the point at which the 1,200 masses became significantly warmer than ambient ($p \leq 0.05$). Larger masses had ADH values that diverged from ambient after as little as ten hours into feeding and development. Preliminary data also suggests that larvae feeding in a mass experience two peaks in temperature, one shortly after reaching third instar, and a second, smaller peak immediately prior to the post-feeding phase of development.

In order to determine whether these differences in microclimate temperature affect rates of development, larvae were sampled from different-sized masses at hourly intervals. This allowed identification of the exact time at which individuals reached specific stages of development, primarily the point at which >50% larvae metamorphose from first to second instar, second to third instar, and then the onset of migration. Preliminary results suggest that there were significant differences ($p \leq 0.05$) when comparing the time (minutes) required for larvae from different-sized masses to reach these developmental junctures. Larger masses appeared to require less time to reach the third instar and post-feeding phase of development in comparison to smaller masses. However, there were no significant differences ($p > 0.05$) when comparing the ADH values for each of the aforementioned developmental points, with all larvae requiring similar levels of accrued heat energy to progress through each stage regardless of their mass size.

These results imply that the thermal output of a maggot mass is dependent on its size and has the potential to affect the rate at which larvae develop. Therefore, by incorporating maggot mass temperatures in the form of ADH values into current methods used to estimate larval age, it may be possible to increase accuracy for PMI estimates in criminal investigations. Further research is encouraged in this area in order to expand on the current understanding of maggot mass thermodynamics.

Maggot Mass, Heat, Larval Development

G156 The Pre-Colonization Gap: When Do Blow Flies First Lay Live Larvae on a Corpse?

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The goal of this presentation is to show how Postmortem Interval (PMI) determination remains the main focus of forensic entomology. However, minimum time frames are based on time of first colonization by the oldest immature found on the body. Following this presentation, attendees will have a better understanding about the question that has always eluded forensic entomologists, which is the period between death and colonization. However, this study also revealed that so-called secondary and tertiary species that visit a corpse are, in fact, primary.

This presentation will impact the forensic science community by determining how soon following death an exposed corpse is colonized by both oviparous and ovoviviparous adult flies, hopefully playing a major role in future PMI determinations.

Forensic entomology is the study of insects within a legal framework. The outstanding variable in determining an accurate PMI is the time that adult flies first lay live larvae or eggs onto a body. The “hinge pin” in forensic entomology is based on the predicted order of insects, typically blow flies and flesh flies, which are attracted to a decomposing corpse or cadaver. Many references have divided the predicted order into primary, secondary, and tertiary insects. This correlates with the process of decomposition and how it changes over time e.g., fresh, bloat, active decay, and skeletal stages. Blow flies generally arrive after death and recent research has shown that *Calliphora dubia*, *Calliphora varifrons,* and sarcophagid adults lay live larvae onto guinea pig carcasses within one hour of exposure. These flies are typically the primary visitors to a corpse. Very little is known about ovoviviparous blow flies, which are invariably the first blow flies to visit a corpse in southwestern Australia and are often the critical species in a PMI determination. New trials exposed 30 guinea pig carcasses throughout the day (0600 – 2000 hours) during spring and summer in bushland on each of five successive days. Replicate carcasses were set up randomly along a kilometer of bushland track and a carcass was removed every 0.5, 1.0, 2.0, 4.0, 8.0 hours throughout the day. Blow flies layed eggs onto carcasses within 30 minutes (33% of carcasses) and, on average, on all carcasses within 1.5 hours of death. Eight different oviparous (including *Lucilia sericata*, *Chrysomya rufifacies*, *Chrysomya varipes,* and *Australophyra rostrata*) and the ovivivparous fly species above deposited either eggs or live larvae onto the carcasses within six hours.

This study highlights the previously unknown rapidity with which blow fly species are able to commence laying onto carcasses and takes into account a large number of fly species, some of which are considered in the literature to be late colonizers of carcasses. A repeat exposure of carcasses again over five successive days in summer revealed five similar species of blow flies laid onto carcasses; however, extreme high temperatures significantly reduced overall blow fly activity. A further trial will be conducted in spring and summer to again evaluate this pre-colonization period. This research has many ramifications for the PMI calculation, especially if all flies

previously designated secondary and tertiary are, in fact, primary visitors to a corpse.

Postmortem Interval, Ovoviviparity, Blow Fly

G157 Characterization of Cypsela-Pappus Units in the Asteraceae: A New Tool for Forensic Botany

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The goal of this presentation is to provide law enforcement personnel and botanists with a means to identify certain types of seeds and their appendages.

This presentation will impact the forensic science community by stressing the importance of forensic botany as a legimitate aspect of criminal investigation, where the smallest plant fragments may provide vital evidence that can help lead to a conviction. The field of forensic botany is slowly gaining recognition as an aspect of criminal investigation that can provide trace evidence previously overlooked either because its importance was not recognized or because investigators did not have the knowledge of how to recognize, seize, and preserve the material for later analysis.

Plant fragments may play a role either in linking a victim and perpetrator, locating a body if it has been moved to a site other than where the homicide took place, or linking either victim or perpetrator to a particular vehicle or location.

Two strategies for seed dispersal by plants are wind and animals. Seeds may be armed with hooks or have roughened seed coats that adhere readily to fur or clothing. Other seeds have tufts of fine bristles, termed a pappus, that aid in wind dispersal but also readily stick to fur or clothing. The daisy family, Asteraceae, is composed of many species that have evolved to use animals, including humans as a means of spreading their seeds, referred to as cypselae, far away from the parent plant. Furthermore, such plants are often found in droughty and nutrient-poor sites such as road sides and other waste places. It is in just such places to which, or across which, a body might be carried or dragged.

The present research explored 21, separate species of the daisy family growing in a single geographic region (Sudbury, Ontario, Canada) to determine if recognizable differences existed between seeds and their tufts of bristles and whether these differences were great enough to allow identification to species.

Cypselae and pappi were examined separately and systematically at increasing levels of magnification to distinguish cypselae and pappi from each other, first by eye, then with a dissecting microscope, followed by a compound microscope, and, finally, under Scanning Electron Microscopy (SEM). Cypselae were distinguished on the basis of size, shape, and surface texture. Under the dissecting microscope alone, it was possible to identify to the level of species in 12 of the 21 cypselae examined.

The pappus was removed from the seed and treated separately because it often becomes detached from the seed head when brushed up against and, therefore, might be the only evidence seized. Intact pappi were separated on the basis of shape, size, and number of bristles using the dissecting microscope. The tuft of bristles was mounted on a microscope slide using glycerin jelly and viewed both under dissecting and compound microscope as was dictated by the level of detail being examined. Bristles were observed in terms of length, bristle width and number of cells, shape of the bristle terminus, and ornamentation of the bristle, such as barbs or spurs. Examination of the pappus alone or its individual bristles made it possible to distinguish six species using the dissecting microscope and eleven species using the compound microscope. The two keys that were devised for separating both cypselae and pappi were submitted to a blind test by three individuals with no previous experience with the material to ensure that the keys were functional and unambiguous.

A small number of species could not be distinguished one from another even when examined under the compound microscope. This group was observed using SEM and an atlas of images made to allow comparisons with unknown specimens.

The field of forensic botany is slowly gaining recognition as an aspect of criminal investigation that can provide trace evidence previously overlooked, either because its importance was not recognized or because investigators did not have the knowledge of how to recognize, seize, and preserve the material for later analysis. The present work indicates that the smallest plant fragments may provide vital evidence that can help lead to a conviction.

Cypsela, Pappus, Asteraceae

G158 Insect Repellent and Insecticide Effects on Adult Insect Activity at Carrion in Northern Virginia

Diana Fleming, MFS, MA, and Elizabeth Richards, PhD*, Travis AFB, AFOSI 1 FIS, 721 Vandenberg Dr, CA 94535*

After attending this presentation, attendees will better understand the potential effects of insect repellents and insecticides on insect activity at carrion and, consequently, on the estimation of a Postmortem Interval (PMI) for human remains by a forensic entomologist. A comparison of the effects of two insect repellents and one insecticide on diptera and coleoptera activity on carrion and relative decomposition of that carrion will be presented.

This presentation will impact the forensic science community by raising awareness of the potential impact the presence of insect repellents and/or insecticides may have on insect activity associated with carrion. Understanding the potential influence such chemicals may have on insect activity and, in turn, decomposition, will be useful to forensic entomologists and the law enforcement community when estimating a PMI. Without understanding the ultimate effects insect repellents and/or insecticides may have, it is possible an approximation of time since death may be under- or over-estimated.

Many factors influence detection and colonization of carrion by necrophagous insects; some factors may accelerate, slow, or even act as barriers to insect activity. Some potential barriers to necrophagous insect activity are natural, man-imposed physical barriers, and man-imposed chemical barriers. This research study addressed three different man-imposed chemical barriers: two insect repellents (a natural oil-based repellent called EcoSmart and a 99% N,N-diethyl-3-methylbenzamide or DEET® spray and one insecticide (Raid®).

In this study, eight skinned, frozen rabbits were utilized. All eight rabbit specimens were of roughly the same size and weight. The specimens were secured inside cages and placed approximately six meters apart on a grassy residential lawn. The chemicals used in this study were EcoSmart®, DEET® spray, and Raid®. Two test groups were utilized: Group A and Group B. Group A tested the effects of the repellents and insecticide in open space. Group B tested the effects of the same three chemicals; however, the rabbit specimens were loosely covered in plastic. For each rabbit specimen in Group B, the outside layer of the plastic was sprayed with each of the three chemicals. All tests were run simultaneously, ensuring equal exposure to temperature changes, minimizing the variables affecting the results. The experiment was structured to test the effect of each chemical by comparing the number of adult flies and adult beetles collected next to each specimen by day. Collection of adult insects was facilitated by the use of sticky rodent traps. Only adult insects from the Diptera and Coleoptera orders were counted. The insects were identified only to order versus down to species and the identification was made visually for the purposes of counting total numbers of adult flies and beetles. Data analysis was conducted utilizing a quantitative versus a qualitative method. The collected data, namely numbers of flies and beetles by each specimen on each day of the experiment, was subsequently utilized to calculate mean values and standard deviation. Mean and standard deviation were

calculated for each rabbit specimen by day, group, type of insect, and combined insect count.

Based on the comparison of the collected data to the normal standard deviation range, significant results were identified for specimens treated with DEET® and Raid® in both groups.

This experiment was successful in demonstrating that DEET® and Raid® can have a significant effect on adult insect activity on carrion and, therefore, may affect the rate of decomposition. While some killers may apply methods to accelerate the rate of decomposition or to prevent a body from being found or recognized right away, altering the rate of decomposition through the application of insect repellents or insecticides could also have a significant impact on investigations. Delayed insect activity on a body could make it appear as if the body has been decomposing for a shorter amount of time than it actually has, thus possibly leading to an inaccurate time frame for events leading up to the discovery of human remains. Further studies are required to test the effect of different insecticides and insect repellents, specifically on necrophagous insect activity. Such studies would greatly benefit investigators and forensic entomologists when an accurate estimation of a minimum postmortem interval (mPMI) and a Period of Insect Activity (PIA) are needed.

Forensic Entomology, Insect Repellents, Insecticides

G159 Phenotypic Plasticity of *Cochliomyia Macellaria Fabricius* (Diptera: *Calliphoridae*) in Texas: How Intraspecific Variation Can Impact Forensic Entomology

Charity G. Owings, BS, Aaron Tarone, PhD, Clifford Spiegelman, PhD, and Jeffery K. Tomberlin, PhD, Texas A&M Univ, College Station, TX 77843*

After attending this presentation, attendees will be more familiar with the concept of phenotypic plasticity, different phenotypic responses of blow flies reared under the same environmental conditions, and ramifications such plasticity can have in death investigations.

This presentation will impact the forensic science community by emphasizing the inherent variability that exists within natural populations of forensically important arthropods.

Phenotypic plasticity, or the ability of a single genotype to produce multiple phenotypes under alternative stresses, has been documented in genetic and ecological literature and spans across many phyla.[1-2] Genotype by Environment interactions (GxE) may influence conspecific populations facing alternative conditions.[3] Given this, subpopulations of forensically relevant insects should also possess the ability to adapt to a shifting environment as exposure to distinct stresses increases. Recent literature in forensic entomology has focused on this phenomenon, particularly in relation to growth substrate and geographic distribution of blow flies (Diptera: Calliphoridae).[4-5] In these studies, development rates of conspecific blow fly subpopulations were significantly different from each other when reared in distinct environments. The implications of plasticity between conspecific blow fly populations lie in estimating the Time Of Colonization (TOC) with published developmental data. If conspecific blow fly populations do not exhibit similar biology but are treated as equals when forming this estimate, less accurate TOC and, thus, minimum Postmortem Interval (mPMI) estimates may be made. These errors may have drastic outcomes, such as a faulty verdict in a court of law.

This study was conducted at the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility on the Texas A&M University campus, College Station, Texas. The goal of this research was to explore variable responses of three conspecific populations of the secondary screwworm, *Cochliomyia macellaria* (Diptera: *Calliphoridae*), in two distinct environments. Phenotypes measured include mean development time (divided into immature and pupal development time), and mean pupal and adult mass. In this research, three geographically distinct populations of *C. macellaria* were reared at 31°C, 70% RH and 21°C, 65% RH. Each temperature treatment contained 15 population replicates, with each replicate housing 100 fly larvae on 50g beef liver. All replicates were placed randomly within two growth chambers and were observed every twelve hours until the wandering third instar stage. At this time, observations were switched to every eight hours. All pupae were collected, weighed, and returned to the appropriate incubator to observe for adult eclosion. Adults were frozen at -20°C, dried at 55°C for 24 hours, sexed, and weighed. Data were not normally distributed and could not be appropriately transformed. Thus, Friedman ANOVA tests and Wilcoxon paired comparisons were used to determine differences between populations and temperature ($p<0.05$).

It was demonstrated that all three strains exhibited genetically different developmental responses for immature and pupal duration ($p<0.0001$). However, unlike the immature stage, responses in the pupal stage were not similar and, thus, showed evidence of adaptation of at least one population to a distinct environment. This is represented by a significant GxE interaction in pupal duration ($p<0.0001$) and pupal mass ($p=0.0232$).

In conclusion, this research demonstrates the importance of considering geographic distribution as a source of variability when estimating a TOC event. In order to comply with the last requirement of the *Daubert* statute (known error rate), the forensic entomologist must have some idea of the variation that a species can exhibit via phenotypic plasticity.[6]

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Plasticity, Calliphoridae, Texas

G160 Analysis of the *De Novo* Transcriptome of Immature *Chrysomya Rufifacies* (Diptera: *Calliphoridae*) to Investigate Sexually Dimorphic Patterns of Gene Expression and Their Role in M-PMI Estimates With a Forensically Important Fly

Meaghan L. Pimsler, BS, Sing-Hoi Sze, PhD, Jeffery K. Tomberlin, PhD, and Aaron Tarone, PhD, Texas A&M Univ, College Station, TX 77843*

After attending this presentation, attendees will gain a better understanding of sexually dimorphic patterns of gene expression throughout the immature development of a blow fly of international forensic relevance. Furthermore, members will acquire insight into

the application of these data to the development of sex-specific growth development curves and molecular methods for estimating ages of immature blow flies.

This presentation will impact the forensic science community by adding to the body of molecular data available for forensically relevant species of flies, which will help in refining time of colonization estimations using insect evidence in immature stages. Currently, the pupal stage is one of the most difficult stages in which to make an estimate of age, due to its externally quiescent nature. While predictable developmental changes are occurring in pupae, a very detailed understanding of fly development is required to make even qualitative refinements of pupal age with morphological data alone. The application of these genomic tools will enable researchers to use gene expression profiles to estimate age more accurately and reliably.

Previous work has identified temporal patterns of gene expression throughout immature development in arthropods,[1] such as Diptera,[2-4] and there is a wealth of research demonstrating sex-specific patterns of gene expression.[5-9] Other work has suggested that, in addition to differences in temporal patterns of gene expression, the sexes of the blow fly *Lucilia sericata* may also develop at different rates (Picard, personal communication). As a result of this, it may be possible to investigate molecular mechanisms governing this plasticity and develop predictive models that incorporate sex, gene expression, and accumulated degree hour data. Though there is a well-annotated and manually curated database, "FlyBase,"[10] of gene expression profiles throughout development in *Drosophila* (Diptera: *Drosophilidae*), a greater breadth of temporal gene expression profiles across Diptera will help researchers to better understand the evolution of developmental pathways and patterns of conservation. Further development of sex-specific differences in temporal gene expression patterns should also prove informative. *Chrysomya rufifacies* (Diptera: *Calliphoridae*) (Macquart) is an especially tractable model organism for these kinds of questions as it exhibits monogenic sex determination with single-sex offspring clutches and homomorphic sex chromosomes.[11-13]

In this experiment, offspring from an isolated female *Ch. rufifacies* were collected and flash frozen. The sex of each larva sampled was determined once the remainder of the cohort had eclosed. For each sex (male and female) and time-point, samples from six separate cohorts were collected. RNA was extracted, sequenced with Illumina HiSeq, and assembled with custom software similar to ASplice.[14] Analysis of the transcriptomes yielded putative markers of the stages of interest. The most promising markers were verified by designing primers based on predicted transcripts and testing them via qPCR.

Many of the genes with homology to those found in other organisms exhibited differential expression profiles across immature stages in accordance with patterns identified in *Drosophila*, especially in highly conserved genes. However, there was greater homology with the more closely related *Lucilia sericata* transcriptome assembled in 2010.[15] The sex-specific markers from previous research were also observed in the immature stages.

In conclusion, genomic tools can be used to improve precision and accuracy of mPMI estimates derived from this species. In addition, this study demonstrated that this species of fly also has sexually dimorphic temporal patterns of gene expression throughout immature development, and that these genes share some homology with those found in other organisms. Furthermore, this work suggested that males develop slightly faster than females, and highlight a need for the inclusion of the sex of the individuals in creating developmental profiles. As the insects age, there is a concurrent increase in variation in terms of the relationship between size or length and time. The development of sex-specific growth curves would provide one additional explanation of that variation and, therefore, increase estimate precision.

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Forensic Entomology, Transcriptome, mPMI Estimate

G161 Triple Primed PCR for SE33 Allele Sequence Determination

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After attending this presentation, attendees will understand what the Triple Primed Polymerase Chain Reactiom (TP-PCR) method is, how it works, and how it can be used to sequence alleles of the SE33 locus. Attendees will also have a better understanding of the discrimination power of the SE33 locus.

This presentation will impact the forensic science community by presenting a new technique to determine allele sequences without Sanger sequencing. TP-PCR is an inexpensive, sensitive and fast alternative for allele sequencing. The application of this method will

enhance the discrimination power of the most polymorphic marker in current Short Tandem Repeat (STR) kits.

The locus SE33 is currently the most polymorphic STR in forensic DNA kits. The alleles of the SE33 locus are characterized by having a typical four base-pair (AAAG) length variation as well as an additional sequence polymorphism due to a single hexanucleotide (AAAAAG) insert that occurs once within the repeat region. This complex repeat pattern is represented as $[AAAG]_x$ AAAAAG $[AAAG]_y$. Because of this complexity, the 70 different length alleles can have up to 13 different sequence variations for a total of 171 alleles. But, the locus-specific primers (P1, P2) of the conventional Polymerase Chain Reaction (PCR) method utilized in forensic DNA typing can only distinguish length variation, limiting the discrimination power of this locus.

Previously, the only method to determine allele sequence was Sanger sequencing, an expensive and time-consuming process. This study demonstrates a novel method for allele sequence determination of locus SE33 using the TP-PCR. This method uses a triad of primers: a locus specific, fluorescently labeled flanking primer (P2), a primer with the repeat unit on the 3' end and a non-binding non-human DNA sequence on the 5' end (P4), and a paired primer of the same nonhuman tail sequence (P3).

During early amplification cycles, the P4 primer anneals directly to the repeat region, producing multiple fragments of varying length. But, the two additional adenine nucleotides of the hexanucleotide unit prevent the P4 primer from binding across this region. This interruption produces a 31 bp gap in the successively longer PCR fragments that correlates directly with the location of the hexanucleotide repeat within the sequence. In later cycles, the paired primer P3 and the fluorescently labeled flanking primer (P2) exponentially amplify the mixture of fragments produced by the P4 primer. This prevents a gradual shortening of the average PCR product due to the P4 primer annealing to sites within the sequences of products of earlier amplification cycles.

Each peak on the resulting electropherogram represents a specific number of repeat units within the SE33 allele. The smallest peak represents the 6 AAAG repeats present in the P4 primer and each successive peak an additional repeat unit. The 31bp gap between peaks separates the fragments produced from the P4 primer binding before and after the hexanucleotide repeat within the sequence. Consequently, by simply counting the peaks on each size of this gap the allele sequence is determined. This study establishes that this technique is powerful enough to differentiate the allele sequences of samples genotyped as homozygous with conventional STR kits. Furthermore, this technique deduced the correct sequence of the previously unpublished 16.2 and 17.2 alleles.

This study demonstrates that TP-PCR is a single reaction method that can be used to maximize the discrimination power of SE33 by allowing allele sequence determination without Sanger sequencing.

SE33, Allele Sequencing, Triple Prime PCR

G162 Application of the IPCRp Method for Genotyping of Male DNA Obtained by Pressure Cycling Differential Extraction

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After attending this presentation, attendees will understand some principles and methods used in comprehensive approach to the problem of obtaining a male genotype from sexual assault evidence where the major issue is the mixture of body fluids from the victim and the suspect. Application of the standard methods on these mixtures often produces an incomprehensible genotype of the suspect.

This presentation will impact the forensic science community by providing attendees with a better understanding of how the small quantities of a male DNA material obtained by the pressure-cycling differential extraction method can be efficiently amplified and detected by IPCRp method (Isolation of PCR products).

The IPCRp method has been previously described to be a fast and efficent way to amplify the low copy number DNA. The method itself increases the sensitivity of the PCR reaction several-fold by highly concentrating a dye-labeled DNA target that is ready to be loaded on the genotyping platform. Therefore, only a few copies of the DNA are sufficient to obtain the genotype. The method utilizes the PCR reaction in which the reverse primer is labeled with biotin acting as a probe for capturing the fluorescently-labeled targeted DNA strand during the amplification. After the PCR, the biotin is attached to the streptavidin-coated plates. Following the washing step, the single-stranded dye-labeled targeted DNA is released by denaturation and loaded for detection on a genotyping instrument. Results are almost free of background noise due to the washing step, in which all of the unincorporated labeled and labeled primers, dNTP's, PCR-reaction buffer, and polymerase are separated and do not interfere with detection.

Control DNA samples prepared by mixing the epithelial and sperm cells and differentially extracted by using pressure cycling technology yielded small quantities of male genomic DNA. The pressure cycling technology followed by the DNA extraction manipulation resulted in a degradation of the female epithelial cells (female DNA). The male DNA was amplified by modified Y-STR and autosomal PCR-multiplex amplification kits. To increase the sensitivity of the PCR, the IPCRp method was applied. The isolation of the PCR products method required modification of the standard multiplex PCR genotyping kits. The amplification kits were designed with the forward and reverse primers fluorescently and biotin labeled, respectively. Following the PCR, performed under standard conditions, the amplified products were captured on the streptavidin-coated, 96-well plates and following the washing step of the unincorporated labeled and unlabeled primers, dNTP's, polymerase, and other material, only the fluorescently-labeled PCR-targeted products in a single stranded DNA configuration were released by denaturation, and loaded into a capillary electrophoresis instrument. Full profiles of the DNA samples were obtained by both kits, although the results from the modified Y-STR kit were with less background noise than the autosomal kit, presumably due to the combined effects of both differential extraction and differential PCR amplification (capturing).

This simple and robust approach of using the pressure cycling technology for differential extraction and decomposition of the epithelial cells, followed by the IPCRp amplification of the male DNA, can improve the genotyping of the male DNA obtained from the mixtures of body fluids from the victim and suspect. Although the IPCRp procedure requires modification of the standard DNA-identification kits, it added only a few extra steps (capturing and washing) to the standard post-PCR protocol which increased the additional time of the post-PCR manipulation by 20 minutes; this is an insignificant accommodation for the standard forensic laboratory practice compared to the benefits of obtaining a genotype of the suspect.

IPCRp, Pressure Cycling, Sexual Assault

G163 Comparative Analysis of Size Specific DNA Concentrators Following Organic DNA Extraction

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After attending this presentation, attendees will understand some principles of DNA analysis, the differences between Sartorius Stedim Vivacon and Millipore Amicon concentrators, and how the addition of PCR inhibitory substances influences the recovery of DNA following

PCIA organic extraction. Attendees will be introduced to principles behind organic DNA extraction methodologies, including the practical benefits of selecting function-specific DNA concentrators. The current study will demonstrate the efficiency of DNA recovery using four commercially available centrifugal DNA concentrators following PCIA organic DNA extraction of forensic type samples.

This presentation will impact the forensic science community by presenting data suggesting the practical benefit of size-specific DNA concentrators for the optimal recovery of DNA from forensic casework type samples. The results of this study demonstrate a significant difference in concentrator brands' abilities to recover nucleic acids, which may cause forensic laboratories to reconsider which concentrator units are currently being utilized.

The use of organic extractions followed by centrifugal concentrators for the purification of DNA is an important tool for forensic laboratories. Centrifugal concentrators both remove PCR inhibitory substances and concentrate the nucleic acid in a sample.[1,2] They are designed to speed up the evaporation of solvents from DNA samples so that purified DNA can be collected and further tested. The objective of the current study was to determine which centrifugal concentrator pore size would recover the most DNA by analyzing samples of whole blood dilutions in deionized water ranging in concentration from 0.2 - 40%. Internal Positive controls for all samples were compared in order to assess the efficiency with which each concentrator removed inhibitory substances.

The purpose of this study was to determine which DNA concentrator (filter and pore size) was better suited for organic extractions based on percent recovery and quality of profile generated. The two DNA concentrator brands under evaluation included Sartorius Stedim Vivacon 500 (size YM-50 and YM-100 Nominal Molecular Weight Limit in Daltons) (Goettingen, Germany) and Millipore Amicon® Ultra (size 50 and 100 NMWL) (Darmstad, Germany). The filter membranes of Amicons have a vertical design allowing for a greater membrane surface area, promising to provide fast sample processing and high sample recovery.[3] The Vivacon filters utilize a horizontal filter design which is equipped with the patented regenerated cellulose Hydrosart® membrane.[4]

In brief, compared to Vivacon filters, Amicon filters demonstrated reduced DNA recovery, on average recovering 52% less nucleic acids. Furthermore, the data indicated decreased amplification efficiency indicating a failure to remove all inhibitory substances. The use of Vivacon 100 NMWL concentrators following PCIA organic extraction has the practical benefit of optimum recovery of DNA and total removal of PCR inhibitory substances, which is particularly advantageous when dealing with forensic samples.

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Forensic Science, DNA Analysis, Concentrator

G164 Evaluating Length Heteroplasmy in the Human Mitochondrial DNA Control Region

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After attending this presentation, attendees will have a better understanding of length heteroplasmy in the human mitochondrial DNA control region. The importance of point heteroplasmy in forensic casework is already known; what is less understood, but more commonly encountered, is length heteroplasmy. The purpose of this research was to examine the mechanisms and mutation rates of human mitochondrial DNA length heteroplasmy, to assist forensic practitioners to identify length heteroplasmy, and to use our guidelines to analyze data where heteroplasmy is present.

This presentation will impact the forensic science community by showing forensic scientists how to analyze mitochondrial DNA results that are complicated by exhibiting length heteroplasmy. Previously, these types of mtDNA results may have been disregarded due to the unknown mutation rate and mechanisms of length heteroplasmy. In this presentation, pedigree data from families in Kerala, India, have been analyzed to elucidate the mutation mechanism and the mutation rates of length heteroplasmy. For the application of length heteroplasmy to forensic science, these results demonstrate the importance of understanding heteroplasmy to be able to confirm maternal relationships, and shows that new point mutations are heteroplasmic in the first generation. Worked examples to assist the forensic practitioner will also be presented.

Examination of the mitochondrial DNA control region found that in more than five percent of mother-child pairs a mutation has occurred in at least one mtDNA C-stretch, blurring the biological relationship between the woman and her alleged child. How then should the forensic practitioner handle fast-mutating C-stretches? This problem was explored experimentally and statistically in five steps: (1) first, DNA samples were collected from 248 families (1,172 individuals) living in the most highly radioactive area in the world (Kerala, India). The natural radiation levels in coastal Kerala are about 10mSv and are, therefore, around ten times higher than normal. This provides an environment which speeds up the mutation rate and allows mutations to be observed as they happen and are transmitted to the children. DNA samples were collected from members of the family covering up to four generations, so mutations occurring between generations could be examined; (2) second, the presence of three known C-stretches and identified a fourth new C-stretch in the mtDNA have been confirmed. The mtDNA control region in humans is approximately 1,122-bp long and is comprised of regions with repetitive tracts of consecutive cytosines. Such "C-tracts" are prone to length heteroplasmy; in other words, one individual can have various mtDNA lengths in his or her cells which differ by the number of C's at these C-tracts; (3) third, a consistent classification of C-stretch status was developed based on the underlying biological process; (4) fourth, the mutation rate observed in the irradiated families was determined, the mutation rate being accelerated and therefore a highly conservative "worst-case" estimate for any situation likely to be encountered in a forensic mtDNA deficiency case context worldwide; and, (5) finally, based on the mutation rate that was calculated, a simple statistical treatment of C-stretch mutations, which will be demonstrated in a worked example is proposed.

Mitochondrial DNA, Heteroplasmy, Radioactivity

G165 An Unusual Case of Strychnine Poisoning

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After attending this presentation, attendees will learn about strychnine (use and prohibition) and, particularly, strychinine poisoning, which is relatively rare because of its prohibition.

This presentation will impact the forensic science community by the histological and toxicological findings in this poisoning case, which police authorities concluded to be suicide , without finding the container.

Strychnine-related death has been described since the 19th-century. This alkaloid was discovered in 1818. Historically, strychnine was used by the Southeast Asian autochthones on arrows. After its discovery in the occidental countries, the substance was used to destroy animal predators. Synthesis has been possible since 1954. Two toxicity mechanisms are described: the tetanising activity and the paralysing action. It is also a part of curare poisons. Its production has been modified by legislation to protect people against accidental intoxications. In 1999, its use was prohibited in France; since this prohibition, strychnine-related death is rare. The most important issue is knowing the origin of the substance.

A 69-year-old man was found dead at home. A letter was found relating familial conflicts. A bailiff had to visit him because of familial conflicts. External examination found no signs of violence. During the autopsy, a pulmonary edema and a blue substance in the stomach were found. Toxicological analysis measured strychnine at 0.29µg/ml in the blood sample. The blue coloration evoked Taupicine®, a rodenticide countaining 10% strychnine. All these elements suggest a strychnine-related death; however, a specific source has not been found by the police. In the medical history, no previous psychiatric disorder was found. Some relatives spoke with him the morning of his death. He did not exhibit suicidal tendencies.

Strychnine induces seizures with normal counsciousness. Death is frequent without emergency care. Accidental, suicidal, and criminal contexts have been described. Strychnine poisoning in children has classically been accidental, whereas, in adults it has been suicidal. Criminal poisoning was described in the 19th-century by a serial killer. It is believed that strychnine could be employed to commit a terrorism attack. Because of its ability to increase pulmonary capacities, strychnine has been used in sports competitions with injections made during the physical activities. Most of the intoxication is secondary to ingestion or inhalation. Sometimes, strychnine is used as a cutting agent in cocaine products. Some topic intoxication is described, too. Various analyses have been developed for quantification such as GC/MS and HPLC.

This case is unusual. It shows a relatively low rate in comparison with the results given in the literature. Histological examination and toxicological findings have permitted the authors to conclude this was a strychnine poisoning. Circumstances of strychnine-related death must be studied because of the rarity of cases. Because of the prohibition in 1999, people cannot get strychnine-containing products. Most often, the products were acquired a few decades before. Also, it seems that strychnine is relatively stable and is active at least ten years or more. In this case, police authorities have concluded it was a suicide, even if there is no psychiatric history and the container of the alkaloid has not been found.

Forensic Pathology, Strychnine, Poisoning

G166 Accentuation of Asphyxial Death by the Background Effect of Chronic Liver Disease, Splenomegaly, and Abuse of Alcohol and Drugs

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After attending this presentation, attendees will appreciate the difficulty encountered in finding a constellation of anatomical findings suspicious of asphxial death in a young person, found supine on the floor of the bedroom, with a history of important medical diseases.

This presentation will impact the forensic science community by highlighting the interplay between problematic scene findings and circumstantial history with important anatomical features. The latter present challenging constellations that could be ascribed to asphyxial death, but the decedent was also a person with established liver cirrhosis, alcoholism, and positive serology for HCV and HIV infections.

The decedent was a known drug abuser and a drug addict. He was placed on a methadone maintenance program. He was also a regular consumer/abuser of alcohol. He showed positive serology for Hepatitis C and HIV viral infections. He was diagnosed with liver cirrhosis and secondary splenomegaly. In addition, he was treated for schizophrenia and depression.

According to the female partner, she woke up partly due to his snoring and partly to step out of the bedroom for a brief interval, less than five minutes, leaving him in bed. Upon her return, he was found supine on the floor and in a collapsed state. His face was getting increasingly blue. She attempted CPR, getting instructions over the phone from a fire brigade officer who made sure she was on the phone carrying out his instructions until the arrival of the dispatched ambulance crew, which arrived within minutes.

The female partner was not trained previously in CPR and it would appear she was trying her best but realized that the deceased was slipping away. Although the ambulance crew sustained CPR until it was clear the decedent was not responding, the clinical impression of the crew was that the deceased was “dead” by the time they got to the scene. It must have been a traumatic experience to the young female partner who allegedly lost two previous male partners while in active relationships with them at different times. Review of a CCTV revealed that, approximately 36 hours prior to his collapse on the floor of the bedroom, the decedent was in an inebriated, drunken state, falling around, and being supported whenever possible by his female partner. A police car picked up both of them and drove them a short distance to the home of a relative less than three minutes away.

There are important anatomical findings identified in the autopsy mainly: florid petechial and punctate serosal; mucosal and intramuscular hemorrhages involving the face, neck, pleurae, lungs, heart, intercostal muscles, and back of the trunk; multiple external contusions that include bilateral peri-orbital contusions and other contusions identified on the head, back of the trunk, and upper and lower extremities; and, an incomplete, non-displaced, focal, small cricoid cartilage fracture, associated with overlying localized soft tissue hematoma, enlarged spleen weighing close to one kilogram, and patchy acute pulmonary pneumonia. In the neck, there were circumscribed, bilateral perivascular (adventitial and peri-carotid arterial hematoma), peri-neural (peri-vagal and peri-sympathetic ganglion hematomata), and intra-neural (intra-sympathetic ganglion and focal intra-vagal) hemorrhages seen. His postmortem blood toxicological analysis showed the following prescribed drug results: free Codeine: 0.17ug/ml, phenethylamine 0.01ug/ml, paracetamol: present, methadone: 0.37ug/ml, eddp (methadone metabolite): present, nordiazepam: present, mirtazapine: 0.08ug/ml and olanzapine: 0.05ug/ml. Ethanol analysis showed blood level of 71mg% and urine level of 168mg%.

The autopsy findings are concerning in that asphxial death, caused by neck and chest compressions, have been seriously considered, notwithstanding the probable underlying contribution and

effect of liver disease on bleeding tendencies that potentially underscores and accentuates the manifestations of impressive hemorrhages. Could the multitude of contusions influence the way the manner of death is considered in this case?

Asphyxia, Liver, Drugs

G167 A Unique Cause of Death in a Double Hot Tub Fatality: Electrocution by Implantable Cardioverter Defibrillator

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After attending this presentation, attendees will understand how to investigate deaths occurring in hot tubs and other closed environments, especially when more than one fatality occurs simultaneously. One must investigate all environmental hazards that might have impacted all of the victims.

This presentation will impact the forensic science community by exposing an environmental hazard that has not been previously reported to cause death.

Although deaths in hot tubs are not an infrequent occurrence, deaths of two or more people in a hot tub or any other closed environment raises suspicion that an external hazard has caused the deaths of these victims. Hazards include electrocution by faulty wiring or equipment, drugs, extreme temperatures, homicidal violence, and poisons such as carbon monoxide in the breathing environment. Examination of the surrounding environment in which the deaths occur as well as the equipment involved is vital in determining the cause of death. Full autopsies with postmortem toxicology are also necessary in determining the cause of death. Identifying hazards in an environment will also enable the removal of hazards and the prevention of further deaths and injuries.

Implantable cardioverter defibrillators are considered life-saving devices because of their ability to detect and then treat ventricular fibrillation by shocking the heart back into sinus rhythm. However, little is known about the effects of these shocks on other people in contact with these patients when their ICDs fire. In certain situations, it is probably hazardous to have skin-to-skin contact with people who are being shocked by their ICDs.

The case presented is that of two elderly people, a husband and wife, who had a daily habit of sitting in their hot tub and were found dead there by neighbors. Both decedents had a history of heart disease. Examination of the scene revealed the hot tub to be in an open area on the lanai behind their house. Inspection of the wiring and the equipment by medical examiner investigators, an electrical inspector, and an expert hired by the next of kin revealed no electrical hazards. The autopsies of the two decedents showed no injuries and both had severe heart disease. Toxicological studies of both victims were negative. At autopsy, the female decedent was found to have an implantable cardioverter defibrillator. It was removed and submitted for query. The query revealed that the woman developed ventricular fibrillation, and, over a short period of time, her ICD delivered four separate shocks without producing cardioversion. No external source of electrical current was detected by the ICD.

Based on the autopsy, circumstances, and scene investigation, it is the opinion that the cause of death of the woman was ventricular fibrillation due to her underlying heart disease. Since it is unlikely that the husband also died of natural causes simultaneously, it is also the opinion that the husband died from electrocution from his wife's ICD while trying to rescue her. Being in water containing an electrolyte solution greatly reduced the electrical resistance of skin-to-skin contact, making a shock from an ICD more hazardous.

In summary, the investigations of deaths occurring in hot tubs require full autopsies and toxicology along with examination of the environment and inspection of the equipment in use at the time of death. In cases involving ICDs, the device should be queried. People in contact with patients with these devices should be aware that they can also receive shocks that can be fatal in some circumstances.

ICD, Electrocution, Hot Tub

G168 The Shaken Baby Syndrome: How Doctors and Authors Can Support the Indefensible

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After attending this presentation, attendees will recognize Shaken Baby Syndrome, and how it can occur in all socio-professions and economics even when the parents are doctors or if the babies are twins.

This presentation will impact the forensic science community by making attendees aware of Shaken Baby Syndrome because the impossible and unthinkable can happen at any time and in all socio-professional contexts.

Shaken Baby Syndrome is known worldwide. Prevention of Shaken Baby Syndrome is the responsibility of the hospital, private doctors, and the media, but also by information applied to diaper packages and baby formula. The perpetrators are usually in denial or find reasons that are refuted by scientific studies, national or international publications, and by common sense! Thus, the courts are becoming more severe against the perpetrators, even if they take into account family background, profession, social and psychological context.

Lesion descriptions are more and more precise and diagnosis requires very specific and powerful examinations (X-rays, radionuclide bone scanning, CT scan, and RMI) which explore organs such as the brain, the eyes, the skeleton, and the cervical cord. It is sometimes possible to date the injuries if they are multiple and spaced out.

The sad originality of this presentation is to present two cases of shaken babies. Detailed examinations of the lesions were performed in each case.

Case 1: A case concerning two-month old twins. It appeared that in the case of the twins, both had severe and multiple injuries from multiple incidences. But lesions were similar with a similar distribution, a comparable chronology, and a similar frequency. These characteristics indicate that the violence was made almost simultaneously in both babies. The progress of both children (each now one-year-old) is currently good, and it is likely that there will be no neurologic complications and abnormalities.

The lesions consisted of:

- bilateral rib fractures
- vertebral compression fractures (dorsal and lumbar)
- forearm bone fractures
- pelvic bone fractures
- lower limb bone fractures
- encephalic hemorrhagic lesions

Case 2: A case of a shaken 8-month-old baby who died from his injuries, which occurred within a professional medical family. In this case, the parents initially attributed responsibility to the 14-month-old brother as well as trivial actions such as changing diapers.

The lesions consisted of:

- bilateral retinal hemorrhage
- acute subdural hematoma (SDH) bilateral, 9mm thick
- diffuse cerebral edema major
- scalp contusions
- 40mm non-displaced skull fracture (occipital)

The adult present on site explained that the trauma occurred after a fall from his height (75cm) onto the floor. Both parents are medical practitioners.

After a review of the recent international literature and the consensus conference from the French National Health Authority in May 2011 about shaken baby and child abuse in general, a parallel is drawn between the lesions found in these babies and explanations will be given for this presentation.

In both cases, the first doctors who treated these children either did not agree on the diagnosis or raised the possibility, especially in the second case, that the accidental cause could not be eliminated.

It is obvious that the validity of the forensic hypothesis is dependent on the quality of the health practitioners, the first medical observations, the forensic pathologist experience and photographs. In these cases, the explanations for the injuries were inadequate and discrepant.

If assessment and investigation of suspected Shaken Baby Syndrome is a multidisciplinary task (health professionals, senior forensic pathologist, psychologist, social worker, police investigators, judges), it is necessary that doctors and health professionals that are in contact with children are aware that with the Shaken Baby Syndrome, and after considering all possible alternative explanations, the improbable, the impossible, and the unthinkable can happen at any time and in all socio-professional contexts.

Shaken Baby Syndrome, Death, Twins

G169 "The Killer B's" – A Case Series of Consumer Product Related Deaths in Young Children

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After attending this presentation, attendees will have a better awareness of consumer product related deaths in young children.

This presentation will impact the forensic science community by presenting a case series of autopsy findings and circumstances related to consumer product deaths in young children (five-years-of-age) over the past 20 years (1992 – 2012) at the Denver Office of the Medical Examiner.

The U.S. Consumer Product Safety Commission (CPSC) estimates that nearly 30,000 deaths in the United States are related to consumer products annually. In 2010, there were an estimated 181,500 toy-related injuries and 17 toy-related deaths in children less than 15-years-of-age. Between 2006 – 2008, there were an estimated 101 nursery product related deaths annually in children less than five years of age.

The CPSC is responsible for protecting the public from serious injury or death from more than 15,000 types of consumer products sold in the United States. The Consumer Product Safety Improvement Act of 2008 was established with the intent of providing stringent oversight on product manufacturing and testing requirements, penalties for violations, whistleblower protections, and a database for product information and consumer reporting. Despite improvements in federal agency oversight, a significant number of deaths are still attributable to faulty or misused consumer products. The majority of these deaths will be, or should be, investigated by Coroner or Medical Examiner Offices.

Presented is a number of selected cases between 1992 and 2012 where the death of a young child (<5 years of age) was associated with a consumer product. The cases include: (1) a 6-month-old infant male who drowned when his Bumbo baby seat tipped over in an adult bathtub. The Bumbo baby seat was first recalled in 2007, and since then, the CPSC has learned of at least 50 incidents in which babies have fallen from Bumbo seats while they were being used on raised surfaces. In all, there have been over 20 reports of skull fractures to infants using the Bumbo baby seat. This has led to a second recent recall of the baby seat in August 2012; (2) a 3-month-old infant girl who drowned when her baby bathtub flipped over in an adult bathtub; (3) a 4-month-old infant male who aspirated a party balloon. According to the CPSC, balloons are the leading cause of asphyxial related deaths in young children. Accidents involving balloons tend to occur in two ways: some children have sucked uninflated balloons into their mouths, often while attempting to inflate them. Some deaths may have resulted when children swallow uninflated balloons they are sucking or chewing on; (4) an 8-month-old male who suffocated in a beanbag chair; (5) a 4 month-old-male wrapped in a "swaddling" blanket who smothered to death; and, 6) a 17-month-old infant male who strangulated to death while playing with a bead maze toy box.

The forensic community has the opportunity to take an active role in protecting the public from consumer product related injuries and deaths through proper reporting, documentation, and public awareness of tragic events resulting from unsafe or misused products.

Consumer Product, Childhood Deaths, Accidental Deaths

H1 The Temporal Degradation of Bone Collagen: A Histochemical Approach to Postmortem Interval Estimation

Amelia M. Boaks, MSc, 107 Neville Park Boulevard, Toronto, ON M4E3P7, CANADA*

After attending this presentation, attendees will gain knowledge regarding the development and application of a simple histological method that selectively stains collagenous and non-collagenous proteins to determine whether a predictive rate of collagen loss occurs over a scale of months and years.

This presentation will impact the forensic science community by providing a potentially useful technique for estimating the Postmortem Interval (PMI) of skeletonized remains over a range of months to years. As collagen degrades over time, the pattern of degradation may be used to predict the PMI of skeletonized remains in forensic cases. The relative simplicity of this procedure and its easily obtainable equipment (already available in many forensic laboratories) potentially allow for the future widespread use of this method in forensic anthropology.

Forensic anthropologists are currently unable to reliably estimate the PMI of skeletonized remains.[1,2] This pilot study was conducted to determine if bone collagen degrades at a predictable rate over time and if so, to determine if this pattern could contribute to the creation of a quantitative method for estimating the PMI. The sample used in this study consisted of one fresh pig long bone and an additional ten pig long bones from subjects with known PMIs ranging from two to twelve months. The method involved the use of a histochemical stain which, when applied to embedded sections of bone, selectively stained collagenous proteins pink and non-collagenous proteins green. Excess stain was rinsed from the section, and the remaining stain was eluted and analyzed using a spectrophotometer at optical density frequencies of 540°nm and 605°nm to yield an Optical Density (OD) value of each dye. A standard curve was created such that a given OD value at a specific frequency would correspond to a known concentration of collagenous or non-collagenous proteins in the unknown section. The ratios of these concentrations were then calculated and statistical analyses were conducted to determine if a significant change or degradation in the ratio of bone protein concentrations occurred over time. Correlation and regression analyses also were performed to determine if the bone protein ratios were significantly correlated with time and, if so, how much of the correlation (or variance in the sample) could be attributed to this correlation.

A statistically significant change took place, with the largest change occurring in the first two months. The Pearson's correlation analyses revealed a significant negative correlation between the ratios of protein concentrations and time; however, the results of the regression analyses yielded only a moderate R^2 value, indicating that while time has some predictive value in determining the age of a bone, additional factors also appear to have a significant effect in predicting the age of an unknown bone.

Via visual assessments of pre-eluted sections, it was evident that the first regions of a bone section to lose collagenous proteins and stain green were the endosteal and/or periosteal regions. The exception to this pattern was found in those bones whose structural integrity had failed and the cortical bone had cracked. In these cases, green staining appeared along the margins of the site of structural failure. Among the modern samples, there was a clear change in the ratio of the pink and green dyes ranging from almost entirely pink in the fresh sections to predominantly green in most of the 10- and 12-month sections.

In summation, while it is apparent that collagenous proteins are significantly correlated with time, other factors also clearly play a role in the rate and process of bone degradation, or diagenesis. As a result, PMI estimations cannot currently be made solely upon the ratio of remaining collagenous and non-collagenous proteins in bone. Rather, further research which monitors the rate and pattern of diagenesis with respect to time and extrinsic factors, such as soil pH values and microbial activity, should be conducted.

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Bone Histology, Collagen, Postmortem Interval

H2 New Methodologies and Protocols of Forensic Identification by Craniofacial Superimposition

Eugenia Cunha, PhD, Univ of Coimbra, Life Sciences Dept, Forensic Sciences Center, Coimbra, 3000-056, PORTUGAL*

After attending this presentation, attendees will become familiar with the issues of Craniofacial Superimposition (CS).

This presentation will impact the forensic science community by showing how Methodologies and Protocols of forensic identification by Craniofacial Superimposition (MEPCROS) will be used by European scientific units; to promote and validate exchange of CS protocols among different European institutions.

One of the most important objectives of forensic anthropology is the determination of a victim's identity, a process that always requires a suspect in order to compare antemortom and postmortem data. Unfortunately, antemortem records are often unavailable and DNA may be impossible in circumstances where, for example, there aren't known relatives of the presumed victim. Moreover, there are situations where many individuals share the same biological profile (e.g., mass disasters, mass graves). In these situations, CS techniques (a forensic identification process where photographs of a suspected victim are superimposed over an unidentified skull in order to establish whether they belong to the same person) have been successfully applied to exclude or establish identity, particularly in Europe. However, no standards exist and forensic experts apply their own approach to the problem based on the available technology and their knowledge and expertise.

The new MEPROCS projects goal is to propose a common European Union (EU) framework to allow the extensive application of CS in practical forensic identification scenarios commonly tackled by European police units. This framework will include: (1) the implementation of an existing semi-automatic method to assist forensic experts in the application of the CS technique resulting in a simple, quick, and systematic approach; (2) the definition of standard protocols within Europe, leading to the objective application of the CS technique in different forensic identification scenarios; and, (3) the specification of a forensic science methodology to provide an objective evaluation of the forensic identification results achieved by CS, avoiding particular assumptions that could bias the process. Hence, the project clearly promotes the validation and exchange of CS protocols and methodologies among different organizations. The particular objectives

of this project concern supporting the development of a trustworthy CS methodological framework by fulfilling requirements covering educational, technical, economic, social, and security aspects.

MEPROCS is a two-year project founded by the EU commission with more than one million euros. It was launched in Mieres (Spain) on March 12, 2012. It is coordinated by the European Centre for Soft Computing. The multidisciplinary list of participant institutions of this network are: the European Centre for Soft Computing from Spain, the Consorzio Ricerca Sistemi ad Agenti (CORISA) from Italy, the European Council of Legal Medicine from Germany, the Physical Anthropology Lab of the University of Granada from Spain, the Israel National Police (Ministry of Public Security) from Isreal, the Forensic Sciences Centre of the University of Coimbra from Portugal, and the Guardia Civil (Ministry of Interior) from Spain. In addition, there are some other institutions, associations, and/or researchers taking part in the network either as associated partners or supporting institutions: Policía Judiciária (Portugal), Council of Forensic Medicine (Turkey), Forensic Anthropology Society of Europe, International Academy of Legal Medicine, International Association of Forensic Sciences, World Police Medical Officers, Department of Forensic Medicine (University of Copenhagen), Forensic Science Programme (Universiti Sains Malaysia), and Laboratory of Forensic Anthropology and Odontology (LABANOF, University of Milan).

The objectives of MEPROCS require bridging the gap between researchers in forensic anthropology and computer science by defining means to foster the dialogue between the different actors involved (technological partners, forensic anthropologists, and end users) across Europe. MEPROCS is an outstanding tool permitting the creation of a coordinated and connected series of activities in order to put the latter tasks into effect. The network is open to incorporate relevant researchers and/or institutions in the field of craniofacial identification in 2012 and early 2013.

Craniofacial, Superimposition, Forensic Anthropology

H3 Case Report of Facial Reconstruction Using the Cranial Forensic Database in Korea

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The goal of this presentation is to report the first Korean casework application of the facial reconstruction method for human identification and to emphasize the importance developing a forensic database for various forensic applications.

This presentation will impact the forensic science community by demonstrating the utility of facial reconstruction methods in Korean casework as well as emphasizing the importance of developing databases for various forensic applications.

Facial reconstruction is a method to help verify the human identity by reconstructing a face from an unidentified skull. The scientific data for facial reconstruction can be classified broadly into two types: soft tissue thickness between bone and skin surface, and facial features such as eye, nose, mouth, and ear. From 2008 to 2011, the Catholic Institute for Applied anatomy constructed a forensic database including basic cranial measurements and facial soft tissue thickness acquired from 3D models of the cranium, mandible, and face using the computed tomography images of 732 Koreans.

This case report is the first attempt to utilize the aforementioned database to develop a facial reconstruction for a slightly putrefied body discovered naked near a mountain located in Yeowol-dong, Ojung-gu, Bucheon-si, on July 1, 2011. The initial examination of the body reported that the soft tissues of the face and left leg were removed and all phalanges were cut off. The autopsy record estimated that the loss of the soft tissues and the phalanges was due to postmortem animal attack and other trauma was not found. The cause of death was unknown. By forensic anthropologic examination, the body was estimated to be an approximately 40-year-old female. After autopsy, computed tomography of the body was taken, then a 3D model of the skull was produced. The soft tissue thickness at 13 midline landmarks and 21 lateral landmarks from females in their forties were selected from the forensic database. The morphology of the nose was estimated using a regression function to predict the location of pronasale and the size of the nose from the measurement of the bony nasal aperture. The other facial features such as the eyes, mouth, and ear were reconstructed by experience with facial reconstruction. Thirteen midline landmarks and 21 lateral landmarks were marked on the 3D model of the target skull using the 3D model modulating program. The guide bars reflecting the soft tissue thickness were added on the surface of each landmark in 3D spaces. The model of the skull with the guide bar was printed into a rapid prototyping model. According to the Neave technique, the expression muscles and the skin were reconstructed on the rapid prototyping model with clay. After clay work, the reconstructed face was scanned with a 3D laser scanner, then the details such as hair style, color of the iris, and texture of the skin were adjusted using the computer. Finally, several pictures of 3D facial reconstructed models were chosen and posted on mass media to find the family of the deceased. The police are now trying to find the family of the deceased using the reconstructed model.

Facial Reconstruction, Forensic Database, Korean

H4 Evaluation of Five Dental Calcification Formulas for Estimating Age in South Africans

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After attending this presentation, attendees will gain knowledge of the reliability and validity of five dental calcification correction factor formulas that are currently used to estimate age among South Africans.

This presentation will impact the forensic science community by providing information on the applicability of dental calcification correction factor formulas on South Africans and evaluating the reliability for scoring dental calcification.

Demirjian is a popular age-at-death method that examines calcification of seven teeth (I1 – M2) in the mandibular quadrant of 20th-century Canadian children.[1] The methodology is based on a dental maturity score which is derived from both the observed tooth stages and the corresponding weights assigned to each tooth. Using the provided percentiles, the maturity score is transformed into an age estimate. Despite some disadvantages, this method is often preferred to Moorrees which examines calcification per individual tooth rather than per quadrant.[2]

In two validation studies on South African children, the Demirjian formula has been shown to be consistently inaccurate.[1] In order to improve both accuracy and applicability, Phillips and Uys established South African correction factors based on the Demirjian methodology.[1,3,4] While these correction factor formula are currently used in South Africa, they have not been tested for either reliability or validity. The purpose of this study is to test both the reliability of the dental calcification method

and the accuracy of the correction factor formula on an independent sample of South African children.

Three hundred and sixty-three panoramic and radiographs (164 males; 199 females) of black, white, and colored groups aged 6 to 10 years were collected both from private dental practices in Pretoria, South Africa, and from the Red Cross War Memorial Children's Hospital in Cape Town, South Africa. Each mandible in the radiograph was scored according to the Demirjian methodology.[1] The Uys (UFBM and UFBF) and Phillips (P.Indian, P.Black, Tygerburg) correction factors were used to provide an age estimation. The Uys correction factors separated into sex groups but not ancestry, whereas the Phillips correction separated into ancestry but not sex.[3,4] Therefore, a total of five different age estimations inclusive or exclusive of either sex or ancestry were compared to true age in all cases.

Statistical analyses included error margin and bias to validate the technique and Analysis of Covariance (ANOVA) to explore differences between males and females. Error margin evaluated differences between estimated and true ages and bias examined whether the estimations were consistently over- or underestimating. For inter- and intra-observer error, ten images were randomly selected and scored by three observers of varying experience.

P.black had the smallest mean bias, mean error margin, and error margin range (-.27 years, .88 years, .006 years – 4.33 years), while P.Indian had the highest mean bias (.84 years), and UFBM had the highest mean error margin (1.1 years). No statistically significant sex differences in error margin or bias were noted for any of the five correction factors (all p-values >0.05). Inter- and intra-observer agreement ranged between 33% – 88% and 73 – 100%, respectively. The most common disagreement was between stages C (crown complete), D (dentine formation), G (root apex open), and H (root complete, apex closed). Low agreement was found with incisor roots; most likely due to poor visibility of these structures in the radiographic images.

While all five tested correction factors may be useful for estimating age in children, applicability of the methods is problematic, especially without known sex or ancestry. While sex was shown to not affect age estimations, ancestry presented with far more variability. P.Black demonstrated the lowest error margin and bias, which suggests this may be the best correction factor to employ.

Though several correction factors have been derived for South Africans none of the equations are predictive. Upon thorough evaluation of the methodologies, it is clear that none of the outputs are presented in a usable fashion; formula should result in an age estimation with associated standard deviations. Although these researchers have collected population-specific data which should yield better results than Demirjian, the data is not presented in a usable format.[1]

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Error Margin, Bias, Validation

H5 Multi-Instrument Geophysical Surveys of Buried Human Remains in East Tennessee

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The goal of this presentation is to provide crime scene investigators and physical anthropologists with innovative and comprehensive information on the identification of clandestine graves and associated evidence using advanced and readily available geophysical instrumentation. This presentation provides a review of current collection strategies and data processing techniques to present flexible and standardized protocols for the identification of this type of geophysical anomaly under controlled environmental conditions. Attendees will be provided with information to determine the utility and applicability of particular geophysical techniques in clandestine grave searches.

This presentation will impact the forensic science community by showing how the search for human remains must incorporate appropriate and methodical approaches that aim to identify evidence while not adversely affecting a crime scene. The methods of archaeology can provide critical tools for the crime scene investigator, particularly those involving the search for buried human remains and associated evidence. This project examines the application of several geophysical instruments to the detection of buried human remains drawing upon a long history of successful use in archaeological fieldwork. A comparative assessment of the use of Ground Penetrating Radar (GPR) and soil resistivity will be presented to demonstrate the most appropriate instrument given important variables of burial depth, time interval, and especially environmental conditions.

Geophysical survey data were collected on burials examined in both forensic and archaeological settings throughout areas of Tennessee. Three historic (and active) cemeteries in varying soil types and surface conditions were surveyed with multiple geophysical techniques including GPR, soil resistivity, and geo-magnetic survey. In addition, data were collected on four burials, created in 2010 at the University of Tennessee Anthropological Research Facility (ARF), that were subsequently excavated in 2012. The archaeological and forensic burials represent varied soil types, differing interment periods, and diverse pit attributes, thereby providing a unique opportunity to examine and compare data collected with several geophysical survey instruments. The resulting data were correlated with available information on the burials and include excavation records, body condition (before and after), time since death, soil properties, pit morphology, and recent weather and soil conditions to demonstrate critical factors and the most appropriate methods to incorporate in geophysical surveys.

East Tennessee has a variety of soil series and soil types, but clayey and loamy soils dominate. These clay-rich soils pose their own set of obstacles and opportunities for the application of geophysical techniques to locate buried targets. Moisture and porosity in these soils have a significant effect on the type of signal return, and these effects that confound geophysical detection can be systematically accounted for only through controlled studies over time. Geophysical data were collected in soils of differing moisture contents with the experimental burials surveyed twice prior to excavation, once under wet and rainy conditions, and again in dryer conditions. While the more recent experimental burials are consistently detected, the range of contrast at the targets was greatly affected by moisture levels. Shallow human burials in the clay-rich soils at the ARF appear as low-resistance anomalies in moist to saturated soils, while the GPR signal return is attenuated or scattered by the wet, clayey soils. When the soils are *saturated*, the GPR depth of penetration is hindered substantially, and this is true for both the 400MHz and the 200MHz systems that the researchers had access to for this study. Clay particles in general, especially when wet, charge differentially and can cause GPR signal

scatter and system noise such that the likelihood of detecting burials with GPR in saturated clay soils is reduced dramatically.

Burials both at the ARF and at historic cemeteries were surveyed using multiple instruments. The comparison of soil resistivity and GPR data in these soils provides further insight into the subsurface effect on radio-wave and electrical-resistance responses under similar conditions. The historic cemeteries provide a large sample of burial contexts spanning more than 200 years of use with varying depths, and accoutrements, and they provide a baseline of comparison for the experimental human burial contexts at the ARF.

In this research, factors of the environment that constrain when and how to use GPR and soil resistivity in a search for clandestine burials are identified. Fieldwork indicates that the incorporation of soil resistivity with the GPR return data (as a multiple-instrument survey) can dramatically increase the chances of characterizing a buried target.

Geophysics, Clandestine Graves, Buried Remains

H6 Bone Histomorphometrics and Sex Assessment

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After attending this presentation, attendees will gain an understanding of bone histomorphometrics as it relates to partial estimation of the biological profile to identify unknown human remains from forensic settings. They will be introduced to the utility of histomorphometrics in the estimation of sex.

This presentation will impact the forensic science community by expanding the knowledge and scope of bone histology to include methodologies in addition to age-at-death estimation techniques thereby strengthening the overall accuracy of the biological profile and, consequently, the likelihood of positive identification.

Histological analyses have proven valuable in discerning fundamental age information about individuals. In fact, bone histology has been utilized to determine age-at-death for over forty years.[1-5] Histological analyses have not particularly focused on assessing sex as part of the biological profile; however, some research has demonstrated the need for sex-specific analyses when developing age-at-death regression equations.[6,7] Studies have also noted that the mean values of specific histological variables are significantly different between the sexes, possibly indicating different rates of bone modeling and remodeling.[6-8] Though past literature has not attempted to assess sex based on histomorphometrics, the finding that differences in bone remodeling do exist and that sex-specific equations to estimate age are potentially more reliable than sex-pooled equations indicate the utility of such methodologies.

This research was performed at the Department of Anthropology's Mineralized Tissue Histology Laboratory and the Forensic Anthropology Laboratory at the University of Tennessee Medical Center. Forty-eight white male and female decedents of known age, sex, and ancestry from the University of Tennessee Medical Center were sampled during autopsy to remove three one-by-one centimeter specimens from the sectioned margin of the left frontal, parietal, and temporal bones. An additional 50 left frontal bone samples were procured from males and females with known demographics from the Tennessee Medical Center. Complete medical histories were available, so retrospectively, if outliers demonstrate differential bone remodeling or atrophy, they could be excluded from analyses.

Bone samples were given a unique numeric, cleaned and dried, and embedded. Three thin-sections were cut for each sample and then ground and polished for analysis. A research light microscope and computer imaging software were used to examine slides at various magnifications; photographic series of the entirety of each thin-section was captured using a mounted digital camera attachment. Prepared thin-section slides were first examined to recognize cellular structures, evidence of remodeling, and the distribution of remodeling prior to quantification of the following histological features: external table thickness, number of secondary osteons, secondary osteon area, secondary osteon perimeter, secondary osteon maximum and minimum diameters, secondary osteon diameter ratios, secondary osteon Haversian canal area, secondary osteon Haversian canal perimeter, secondary osteon Haversian canal maximum and minimum diameters, secondary osteon Haversian canal diameter ratios, number of secondary osteon fragments, osteon population density, and osteon circularity.

This research created a discriminant function analysis based on the collected histomorphometrics to assess sex. A jack-knifed discriminant function analysis was run separately for the frontal, parietal, and temporal bones and then again to include all three bones to determine how accurate the histological features are at "discriminating" between subgroups and predicting which subgroup (male or female) each individual belongs to. Using only the frontal bone to assess sex resulted in a 67% correct classification, the parietal a 52% correct classification, and the temporal a 64% correct classification. When the discriminant function analysis utilized a combination of sub-variables from the frontal, parietal, and temporal to assess sex, it did so with 80% correct classification for females and 90% correct classification for males with an overall 86% correct classification. These results lend credibility to the prospect of using bone microstructure to assess sex.

In conclusion, bone histomorphometrics has potential in not only estimating age-at-death of unidentified individuals, it also contributes to assessment of sex.

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Histomorphometrics, Sex Assessment, Forensic Anthropology

H7 Patterns in Worldwide Craniometric Sexual Dimorphism and Its Importance in Forensic Anthropology

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After attending this presentation, attendees will understand that differing degrees of craniometric sexual dimorphism among world populations can significantly impact sex estimation.

This presentation will impact the forensic science community by serving as a resource for understanding variation in worldwide craniometric sexual dimorphism and thus, considerations for more accurate sex estimation.

Forensic anthropologists frequently encounter individuals from a variety of ancestral backgrounds in American casework as well as global human rights cases and mass fatalities. The degree of craniometric sexual dimorphism varies among different populations and it is especially important for forensic anthropologists to keep these differing degrees of craniometric sexual dimorphism in mind when assessing sex. Guyomarc'h and Bruzek noted that applying Discriminant Function Analysis (DFA) from modern American craniometric data to a Thai sample resulted in poor classification accuracy.[1] The low classification accuracy in their Thai sample is likely a result of differing levels of craniometric sexual dimorphism between populations in addition to different population sex-specific means. Indeed, the sex-only function in FORDISC 2, which pooled samples of males and females from several groups, was removed in FORDISC 3 because Hispanic males were often misclassified as females.[1-3]

In order to analyze the differing degrees of craniometric sexual dimorphism among world populations, 13 measurements were analyzed from a modern Thai sample from Khon Kaen University Hospital (n=114), modern South African blacks from the University of Pretoria (n=84), and modern samples from the Forensic Data Bank (n=695), as well as 26 population samples that include both males and females from the W.W. Howells Data Set (n=2412). The statistical program R was used to run analyses of the craniometric data.[4] The pairwise Mahalanobis distance (D^2) was used as a measure of the difference between males and females within each group in order to isolate sex differences from ancestry differences, in contrast to pooling all groups in canonical variates analysis, where sex and ancestry influence the differences simultaneously. The classification percentages for males and females were calculated in FORDISC 3.1 for each group. The Easter Island group had the highest classification accuracy (96.5%) indicating a high degree of difference between males and females, making it easier to differentiate between the sexes. The Thai group had the lowest classification accuracy (72.8%), meaning that the males and females were more similar to one another and more difficult to differentiate. That is not generally true for all Asian populations, which are quite variable in the degree of craniometric sexual dimorphism. For example, the Ainu group had a classification percentage of 92.1% while the Atayal had a classification percentage of 74.5%.

The Thai and South African Black groups were the least sexually dimorphic in both the facial and vault measurements while the Easter Island sample was the most sexually dimorphic in both the facial and vault measurements. This shows that a small male from a group such as the Thai could easily be mistaken for a female of another group, such as Easter Island. In general, as the degree of sexual dimorphism in the face increased, the degree of sexual dimorphism in the cranial vault also increased.

The results of this paper further indicate that there are craniometric trends across nearly all samples from around the world. Using stepwise selection in FORDISC 3.1, the first three measurements of the 13 considered that were most important in the discrimination between males and females were related to cranial lengths (glabello-occipital length, nasio-occipital length) and facial width (bizygomatic breadth).

In conclusion, these findings confirm the importance of understanding that levels of sexual dimorphism vary between populations and this can help us interpret classification percentages in DFA and appreciate that sex assessment may be more difficult in those populations where males and females are more similar, such as the Thai, or where smaller males of one group may be confused for females of another group. With more investigations in many parts of the world, the challenge of population-specific methods becomes more important and this study functions as preliminary research into this question in order to formulate more accurate sex estimation in forensic anthropology.

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Sexual Dimorphism, Craniometrics, Sex Estimation

H8 Sex Estimation Using Non-Metric Traits in Thai Crania With the Walker (2008) Method

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After attending this presentation, attendees will: (1) be informed about the current research into sex estimation in the Thai; and, (2) understand the reasons why population-specific methods of non-metric sex estimation are important.

This presentation will impact the forensic science community by demonstrating that a commonly used North American method may not perform well when applied to other populations, and by furthering the research into sex estimation in the Thai.

Non-metric methods of sex estimation are widely used by forensic anthropologists across the world because of their ease of application and usefulness on fragmentary remains. Recently, statistical techniques such as discriminant function analysis have been applied to several well-established non-metric trait scoring systems in order to increase their classification accuracy and reliability, as well as make them admissible in court under the *Daubert* standards. It is also important that population-specific methods of sex estimation be developed, as patterns of sexual dimorphism vary extensively worldwide.[1-3] A method that accurately separates sex in one population may not be as effective in a population that is very different morphologically. There has been little effort to develop non-metric methods to estimate sex in the Thai or other Southeast Asian groups, and those that have been developed lack the integration of statistical methods necessary for reliability.

A non-metric method of estimating sex from crania that is commonly utilized in North America was published and developed using samples of African and European Americans, American Indians, and English.[4] In this study, Walker's method was applied to a sample of 70 males and 26 females from the Kohn Kaen University Hospital in Kohn Kaen, Thailand. Walker's reported logistic discriminant functions were applied to the Thai sample to test their performance. Next, in order to describe differences between the Thai sample and Walker's samples, the means of the samples were compared using Wilcoxon's rank-sum test, and the trait distributions of Walker's samples were compared to the Thai sample using the Freeman-Halton test. Univariate statistical analysis was run using each of Walker's five cranial traits to determine each trait's usefulness in estimating sex in Thais. The Thai sample was also classified through linear discriminant function analysis, using FORDISC 3.1.[5] Finally, the Thai sample was classified with logistic discriminant function analysis.

Results showed that Walker's method is not suitable for use in Thai populations. When Walker's logistic discriminant functions were applied to the Thai sample, their performance was very poor, exhibiting consistently low classification accuracies and high sex biases. In some cases, these functions produced classification accuracies of less than 50%, which is less accurate than random assignment of sex. Wilcoxon's test and the Freeman-Halton test indicated that there were significant differences in trait score means and distributions between the Thai

population and Walker's reference samples. In certain traits, the Thai male sample was significantly different from Walker's male samples, while not significantly different from Walker's female samples. When linear discriminant function analysis was used on the Thai sample, classification rates were consistently lower than those obtained in Walker's study. Using logistic regression derived from the Thai sample did not improve results. Throughout this study, no analysis produced a classification accuracy greater than 80% in the Thai sample.

Walker's method should not be used on the Thai, and must be used with great caution in untested populations as its results may be invalid. Patterns of sexual dimorphism in the Thai are different enough from those seen in Walker's samples that the equations developed by Walker do not effectively classify the Thai by sex. All analyses using Walker's traits performed poorly, even when newly calculated using the Thai as a reference sample; this indicates that Walker's system is not useful for estimating sex in the Thai, due to a lower degree of sexual dimorphism in these traits. New systems utilizing different traits should be explored in order to find those that may be the best for estimating sex from Thai crania.

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Sex Estimation, Non-Metric Traits, Southeast Asia

H9 An Evaluation of the Hartnett-Fulginiti Method for Age-Estimation on an Independent Skeletal Sample

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After attending this presentation, attendees will learn about an evaluation of the Hartnett-Fulginiti (H-F) method for estimating age-at-death.[1-3]

This presentation will impact the forensic science community by providing data concerning the efficacy of this method when applied to a known-age skeletal collection differing from its reference sample. Validation using an independent sample is essential given that age-estimation techniques impose the age structure of their reference sample skeletal to the samples under investigation.[4-5]

Accurate estimation of age-at-death is crucial for the development of biological profiles and, therefore, a foundation of forensic anthropology. The H-F method revises two of the more widely used methods for age-estimation: the Suchey-Brooks (SB) method employing pubic symphyses and the Işcan and Loth (IL) method utilizing the sternal end of the fourth rib. These revisions were based upon a sample of elements from 630 individuals curated at the Maricopa County Forensic Science Center in Phoenix, Arizona. The sample is predominately of European ancestry, and ranges from 18 to 99 years of age. The current research aims to assess the utility of the H-F revisions on a skeletal sample independent of its reference sample as well as its efficacy on a subsample of individuals of African-American descent.

This research employs a random sample of 200 individuals of known age and ancestry from the Hamann-Todd collection curated at the Cleveland Museum of Natural History. The sample consists of 39 females and 161 males ranging between 19 to 87 years of age. Nearly half of the sample is of African-American descent (n=90). Rib ends were unobservable for some individuals and so the H-F rib method was evaluated on a subsample of 182 individuals. Age-at-death was estimated for each individual by examining the pubic symphyses and rib ends and assigning them to one of the seven H-F phases. Spearman's rank correlation coefficients were calculated between estimated and actual H-F phases and the percentages of individuals whose known ages fell within their estimated H-F phase or an adjacent phase were calculated.

Results suggest that the H-F method performs slightly better than the SB and IL methods. Higher correlations were recorded between estimated and actual H-F phases than for similar evaluations of the SB and IL methods (H-F pubic: Spearman's ρ=0.768, p-value <0.001; SB: Spearman's ρ=0.699, p-value <0.001; H-F ribs: Spearman's ρ=0.771, p-value <0.001; IL: Spearman's ρ=0.720, p-value <0.01).[1] The H-F method estimates the correct age phase for a similar percentage of individuals as the SB and IL methods (H-F pubic=41.5%, SB=44%; H-F ribs=41.2%, IL=35%) and it estimates the correct age within one phase on a moderately larger percentage of individuals (H-F pubic=92%, SB=83%; H-F ribs=90.7%, IL=79%).[1]

When the males from the sample are segregated by ancestry, a higher correlation exists between estimated and actual H-F phases for individuals of African-American ancestry than for those of European ancestry (African-American pubic Spearman's ρ=0.770, p-value <0.001, European pubic Spearman's ρ=0.651, p-value <0.001; African-American rib Spearman's ρ=0.812, p-value <0.01, European rib Spearman's ρ=0.625, p-value <0.01), suggesting that the H-F method performs well on individuals of African-American descent despite being developed from a primarily European sample. Data from the female subsample suggest a similar pattern, but sample sizes are too small to permit statistical verification.

Twenty individuals were selected for re-evaluation to assess the degree of intra-observer error. Correlations between first and second age estimates were very high (pubic: Spearman's ρ=0.934, p-value < 0.001; ribs: Spearman's ρ=0.975, p-value <0.001), exceeding those reported for similar tests of the SB and IL methods.[1]

The results of this evaluation of the H-F method for age-estimation suggest that it performs as well as or better than the widely accepted SB and IL methods. Its performance on a subsample of individuals of African-American ancestry indicates that it is unhindered by the primarily European composition of its reference sample. Lastly, the H-F method exhibits low intraobserver error rates. These observations suggest that the H-F method can be employed consistently and fruitfully by forensic anthropologists.

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Pubic Symphysis, Fourth Rib, Age-Estimation

H10 A Re-Examination of Age-at-Death Estimation From the Human Sacrum

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After attending this presentation, attendees will have a more comprehensive understanding of the human sacrum as an age indicator and statistical techniques used to assess age.

This presentation will impact the forensic science community by providing further investigation into traits and statistical techniques for estimating age-at-death from the human sacrum.

The goal of this presentation is to inform attendees about the performance of seven traits previously proposed as significant markers in the assessment of age at death from the human sacrum.[1]

Accurate age-at-death estimation from human skeletal remains is critical for establishing a comprehensive biological profile of an unknown individual, in order to facilitate the victim identification process. While many regions of the human skeleton have methods available to assess age at death, the sacrum has only recently been investigated.[1] The study builds on that previous work.

The primary aim of this study was to assess the performance of seven sacral traits, previously proposed to have utility in the assessment of age-at-death from human skeletal remains. These traits were: fusion of S1/S2, fusion of S2/S3, surface change, apical change, fusion of annular ring of S1, microporosity, and macroporosity. The study sample consisted of n=633 sacra from the Hamann-Todd (n=386) and Bass (n=247) collections. Individuals from the Hamann-Todd collection consisted of males and females, mainly of African and European ancestry, ranging in age-at-death from 10 to 96 years, and individuals from the Bass collection consisted of males and females of European ancestry, ranging in age-at-death from 16 to 97 years. Previous research found no significant sex or ancestry differences in regard to age changes in the sacrum.[1]

These traits develop over a continuum and each morphological character was scored according to multiple trait variants as described in Passalacqua (2009). In order to limit observer error; however, these traits were re-scored on a presence/absence or an unfused/fused scale. Age ranges were arbitrarily assigned into three broad age categories: young adult (<31 years), middle-aged adult (31 – 50 years), and old adult (51+ years). Each of these age ranges were treated as "populations" and subjected to multinomial regression analysis, random forest modeling, a naïve Bayesian model, and linear discriminant function analysis. Lastly, frequencies for each trait within each age group were tabulated and used to calculate Principal Component (PC) scores. The PCs were plotted against themselves to investigate the interplay of each trait in the estimation of age.

Each of these statistical methods for group membership had a similar correct classification rate, ranging from 69.8% correct using a naïve Bayesian analysis to 67.1% correct using discriminate function analysis. This trend, however, did not hold true for classifications of particular age ranges. By far, the most mis-aged age range was the middle-aged adults (31 – 50 years), with three of the four analyses correctly classifying 3.4% or less of this age range. Discriminate function analysis showed a much better ability to correctly classify middle-aged adults, with 38.1% of this group correctly classified.

Principal component analysis showed that the frequency distribution of these traits varied by age. Two principal components were derived from these data. The first PC showed the greatest loadings in the fusion of the S1 annular ring and then by the depression/resorption of the S1 annular ring, while PC2 demonstrated the greatest loading was macroporosity followed by surface changes. When plotted against themselves, the principal components showed a clear separation of each of the age ranges. On PC1, the decades moved in gradual order from youngest to oldest, while PC2 showed the <31-year group and 51+ years in a gradual order while 31 – 50-year group was separated.

Results demonstrated that treating age groups as populations yielded total correct classifications greater than pure chance. In each analysis, middle-aged adults misclassified most frequently. Conversely, the oldest age group (51+ years) demonstrated the highest classification accuracies. The principal components indicated that fusion of the S1 annular ring, depression/resorption of the S1 annular ring, macroporosity, and surface change accounted for the majority of variation present in the sample. Similar to other degenerative aging techniques, the sacrum predictably changes throughout life with younger and older individuals classifying correctly more often than middle-aged individuals.

Reference:

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Forensic Anthropology, Age-at-Death Estimation, Sacrum

H11 Multi-Isotope Study of Modern Human Dental Enamel From a Dutch Population

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After attending this presentation, attendees will understand the potential of combining isotope ratios for different elements in dental enamel to assist in estimating the origin of unidentified victims.

This presentation will impact the forensic science community by providing insight into the use of isotope ratios to assist in estimating the origin of unidentified victims.

Isotope ratio analyses (δ18O, Sr and Pb) have been carried out in wisdom teeth (third molars) from Dutch individuals that were born and lived in The Netherlands (30 individuals) and from individuals (five individuals) that were born abroad (South Africa, Surinam, Germany, Poland, Kenya), and moved into The Netherlands during childhood or adulthood. Also included are results for four front teeth (central and lateral incisors) from two young children (five to six years old), who were born and live in The Netherlands. Results demonstrate the use of isotope ratios for estimating the origin of unidentified human victims.

Variations in radiogenic isotopic systems (Sr and Pb) in wisdom teeth from the foreign individuals were compared to the variations of the Dutch population. The combination of δ18O stable isotope ratios with radiogenic isotope ratios, such as Sr and Pb provides provenance information from different perspectives: For isotopic studies of dental enamel, δ18O provides information on the isotope composition of waters ingested by the individual, the Sr isotope composition links the individual to the geological environment, and Pb isotopes reflect exposure of individuals to Pb sources present in the environment. The multi-isotope method has great potential application in forensic sciences.

The average δ18O isotope composition of dental enamel from the collection of third molars from modern Dutch individuals is 25.62 ± 0.41 (1σ) ‰. The individual from South Africa has a similar δ18O isotope composition compared to the Dutch dental enamel. This is in accordance with the information provided for the South African individual who moved to The Netherlands at two years of age, which is before the formation of third molars that begin to calcify at seven to nine-years-old.

From the δ18O isotope composition of dental enamel, the δ18O isotope composition of the water ingested by the individuals has been calculated using the equations of Daux et al.[+] and Chenery et al.[1,2] The calculated δ18O isotope compositions of the ingested water by the different individuals clearly indicated variable isotopic compositions that depended on the place of origin.[1,2]

Srontium isotope ratios in dental enamel show large variations (0.707 – 0.710) in teeth from Uganda and Surinam due to the different geology (tertiary sediments and volcanics to granitic metamorphic rocks, respectively) in these areas. The Sr isotope results in dental enamel from the Dutch population vary between 0.709 – 0.710, which is comparable to the Sr isotope values in scalp hair, water, and soils in The Netherlands. The combination of Sr isotopes with δ18O isotope composition show distinctive compositions related to place of origin. By only using one isotopic system; for instance δ18O in dental enamel from Uganda and Surinam or Sr isotopes in tooth enamel from Poland and

The Netherlands, the place of origin could not be distinguished. Similarly, Pb isotope results in dental enamel will be discussed.

References:

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Teeth, Isotopes, Human Provenancing

H12 Analysis of Non-Human Skeletal Material Received in a Medical Examiner Setting

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After attending this presentation, attendees will gain new perspectives on the types of non-human skeletal material commonly received from law enforcement for forensic analysis in Massachusetts.

This presentation will impact the forensic science community by providing attendees with the species and taphonomic alterations common to this region and therefore the types of training which should be emphasized in these fields. Previously published documentation of types of non-human cases is sparse and is usually limited to general statements of what species are commonly examined. Forensic anthropologists have noted that commonly examined non-human remains throughout the U.S. are those related to food production such as deer, lamb, pig, cow, and chicken, as well as those from dog and horse. This study seeks to provide an organized and systematic analysis of a large sample of these cases.

Non-human skeletal elements were analyzed by the forensic anthropologist for Office of the Chief Medical Examiner. Cases were from the entire state of Massachusetts and the vast majority were found by members of the public and reported to law enforcement as potential human remains. Information was recorded on the different taxa present, the specific types of skeletal elements, and the different taphonomic processes affecting the bones (such as animal gnawing, weathering stage, and signs of peri-mortem butchery). Remains were analyzed through physical, macroscopic examination, and through photographs.

The most commonly analyzed non-human remains were those of white-tailed deer (*Odocoileus virginianus*), cattle (*Bos taurus*), and pig (*Sus scrofa*). Approximately 40% of the cases analyzed contained at least one skeletal element that showed signs of modern (machine) butchery. Most cases of machine butchery were skeletal elements from *O. virginianus*, *B. taurus*, and *S. scrofa*. This study validates the assessment of species related to food production being commonly presented by the public as potential forensic cases, while dog (*Canis familiaris*) remains were present in only 6% of cases and only one case involved definite horse (*Equus caballus*) remains. Only 5% of cases involved cranial elements, and most of these were fragmented. This most likely can be attributed to the general public's knowledge of the shape of the human skull as a popular symbol, making it difficult to confuse with a non-human cranium, and the active collection of non-human crania when encountered, leaving the more difficult to identify postcranial remains for later discovery. Gnaw marks were seen on 33% of the bones and were categorized as coming from large carnivore, small carnivore, undetermined carnivore, large rodent, or small rodent. Large rodent gnawing consisted of teeth marks left by North American porcupine (*Erethizon dorsatum*). Approximately half of the skeletal elements were at weathering stage 0, with some examples of weathering stage 1, but no cases were noted as being beyond that stage. Due to the state's extensive coastline, there also were cases involving skeletal elements from marine life including elements from dolphin (Delphinidae), seal (Phocidae), and unknown large fish species. Several of these elements had alterations typical of marine coastal environments, including rounding and bleaching.

The compilation of this information can help forensic anthropologists better understand what types of cases are being reported by the general public as potential forensic cases, and the skeletal recognition of which taxa in the North Atlantic region should be emphasized in forensic curricula.

Non-Human Remains, Forensic Anthropology, Taphonomy

H13 Sex Estimation in Modern African and Diaspora Populations

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After attending this presentation, attendees will become familiar with cranial and postcranial metric sex estimation techniques in a modern African population and United States diaspora populations. Prior analysis looking at populations throughout Africa shows strong regional variation, especially concerning sexual dimorphism, which supports implications for using population-specific estimation parameters.

This presentation will impact the forensic science community by offering research into human skeletal variation among under-studied populations.

The estimation of sex is a significant component to the biological profile created by forensic anthropologists during casework. Many methods related to the biological profile have been developed based on skeletal collections in the United States. As forensic initiatives have increased throughout the continent of Africa, questions arise as to whether estimation parameters created from one population are appropriate for diverse groups. This presentation explores this question by using cranial and postcranial data collected at the Raymond A. Dart Skeletal Collection in Johannesburg, South Africa. For this research, a modern Botswanan sample was used containing 31 male and 31 female individuals. The age, sex, and population is known and confirmed for all of these individuals. As a preliminary analysis, by using these Botswanan individuals to create sex estimation techniques to be used in that region of Africa, comparisons can then be made to modern United States samples to assess the variation and determine whether population specific methods are needed.

Both cranial and postcranial measurements were used to create univariate and multivariate sex estimation techniques. All cranial data were collected with a Microscribe G2X digitizer. Seventeen measurements were used. Additionally, 31 postcranial measurements were taken from each individual. Multivariate analyses were performed on the cranial and postcranial measurements in SAS 9.1.3, consisting of a stepwise Discriminant Function Analysis (DFA) to determine which variables are the most accurate when estimating sex. From these variables, classification functions were created for multivariate sex estimation. A linear DFA allowed for sectioning points and cross-validation classification rates to be produced for the cranium and postcranial skeleton.

The multivariate results suggest that the femur's stepwise selected variables allow for a classification function with a total cross-validation rate of 90.53%, which is higher than any of the other classification rates, with the cranium (90.32%) and the humerus (88.89%) following closely behind. When considering only the univariate sectioning points of the postcranial skeleton, the top three total classification rates are measurements of the humerus, including humeral head diameter (88.89%), humerus epicondylar breadth (87.29%), and humerus proximal epiphyseal breadth (87.04%). When utilizing the Robert J. Terry Collection to represent an African diaspora sample, univariate maximum length measurements of the arm also consistently had the highest classification rates with ulna maximum length (83.71%) and humerus maximum length (82.03%). By comparing these resulting measurements and classification rates to African American samples,

insights can be obtained into human variation in Africa and other diaspora populations.

Human Identification, Sex Estimation, African Populations

H14 Evaluating Methods for Measuring the Ischiopubic Index for Determining Sex and Ancestry From the Human Skeleton

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After attending this presentation, attendees will better understand the validity and utility of recently proposed measurement techniques for both the ischium and pubis that differ from Washburn's original method, including several new measurements of the coxa designed by the authors.

This presentation will impact the forensic science community by helping to improve the way that the Ischio-Pubic (IP) Index is used in the determination of sex and ancestry of unidentified skeletal remains. Furthermore, it will illustrate the importance of carefully examining the sources of variance in a skeletal sample to tease out potential environmental effects.

Osteometric techniques employed in studies of the human pelvis are firmly rooted in the defining work of a few individuals, such as Washburn.[1] Unfortunately, Washburn's widely-used pubis and ischium lengths have increased potential for intra- and inter-observer measurement error because the fusion point of the three major elements of the os coxa (ischium, ilium, and pubis), from which ischial and pubic lengths are taken in adults, is often ambiguous. Few studies have attempted to alleviate this problem and those that have, such as Patriquin's recent work, lack validation.[2] Furthermore, few have simultaneously addressed ancestral variation in the anterior pelvis that could confuse the issue. The current research addresses these problems and suggests that a shift to a more precise method for calculating the IP index is warranted.

Six measurements consisting of three pairs of corresponding pubic and ischial lengths were taken using Washburn's, Patriquin's, and a newly developed technique that uses the very center of the acetabulum (the "acetabular point").[1,2] A sample of 220 identified skeletons was drawn from the collections at the University of Pretoria in South Africa and the University of Tennessee at Knoxville. Measurements were taken from males and females of "Black" and "White" ancestry born in both North America and Africa. "White" and "Black" are used in this context in the traditional sense, referring to individuals whose ancestors were previously derived from either indigenous European or indigenous African populations, respectively. All individuals were adults between the ages of 18 and 99 and free of pathological conditions and postmortem degradation.

Each index was calculated using the traditional formula ((pubis length/ischium length) x 100). A fully-factorial univariate Analysis of Covariance (ANCOVA) was applied to each of the three indices to determine whether any of the independent variables (sex, ancestry, age at death, and continent of origin) or their interactions have a significant effect on the variance of each index. Then the accuracy of each index was assessed by generating separate discriminant functions for sex and ancestry and resubstituting the entire sample into each equation, producing percentages of individuals correctly assigned by each method.

The results indicate that all three indices are significantly affected by sex, two are affected by ancestry, and none are affected by age at death. The newly-developed acetabular index has the best accuracy when predicting sex (87% correct) but the worst accuracy when predicting ancestry (53%), suggesting that its lack of sensitivity to ancestral variation allows it to hone in on sex differences more effectively. Patriquin's index had the best accuracy for predicting ancestry (64% correct) but the worst accuracy for predicting sex (78%). Continent of origin appears to have an independent influence on anterior pelvis morphology beyond the effects of sex or ancestry, suggesting that the environment is an important factor irrespective of genetics.

References:

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Ischio-Pubic Index, Sex Determination, Predicting Ancestry

H15 The Birds and the Bones: Differential Avian Scavenging Patterns in Determination of Postmortem Interval

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After attending this presentation, attendees will understand the importance of avian scavenging patterns in the taphonomic process and in determining postmortem interval, as well as recognizing a wider range of scavenging bird species and the marks they leave in skeletal material.

This presentation will impact the forensic science community by illustrating the presence and use of differential taphonomic markings left by avian scavenging, which will provide valuable information about postmortem interval and the taphonomic process.

Postmortem interval is measured largely by taphonomic processes, in many cases specifically by insect scavenging activity. Similarly, scavenging by mammals (most notably carnivores and rodents) and avians has been noted and studied in taphonomic and postmortem interval contexts. However, of the eight avian species known to scavenge on the carrion of large animals (including humans) in North America, only two species have had their scavenging patterns studied in-depth. Reeves studied "the accelerated rate of decomposition and the signature markings on the bones" of pig carcasses (*Sus scrofa*) that were scavenged upon by Turkey Vultures (*Cathartes aura*) and American Black Vultures (*Coragyps atratus*).[1]

This study investigates specifically the markings on bones left by the American Black Vulture, the Andean Condor (*Vultur gryphus*), the Bald Eagle (*Haliaeetus leucocephalus*), the Chihuahuan Raven (*Corvus cryptoleucus*), the Common Raven (*Corvus corax*), the Golden Eagle (*Aquila chrysaetos*), and the Turkey Vulture, all of which were housed at the Phoenix Zoo. The Phoenix Zoo does not display all eight species of known North American avian scavengers, including the American Crow (*Corvus brachyrhynchos*) and the California Condor (*Gymnogyps californianus*). Consequently, the results from the Andean Condor are assumed to be representative of the California Condor and the results from the two Raven species are assumed to be representative of the American Crow, due to similarities in their respective sizes, natural habitats, and diets. All of the species were kept in separate enclosures, with the exception of the Turkey and American Black Vultures, which share an enclosure. This ensured a controlled environment free of terrestrial and overlapping avian scavenging which could potentially confuse resulting data. Every species was represented by two birds, except for the Turkey/American Black Vulture population, which were represented by four Turkey Vultures and three American Black Vultures. Over a one-month period, the birds were first given ham hocks (*Sus scrofa*) for two weeks followed by pork ribs and beef ribs (*Bos primigenius taurus*) for two weeks. The specimens were left in the enclosure for several days until completely de-fleshed or until the next scheduled feeding (approximately every two to three days). The bones were then collected, macerated at the forensic anthropology and odontology laboratory, and examined grossly and microscopically.

No visible evidence of scavenging was found on the ham hock bones that were placed in the enclosures during the initial phase of the research. The pork and beef ribs, however, yielded multiple notable results. Grooves and scratches were noted among the specimens recovered from the Bald Eagle, Raven, and Turkey/American Black Vulture exhibits. None of the observed marks appeared to be consistent

with rodent scavenging, implying that only avian scavenging marks were present. The marks observed on the specimens provided to the Turkey and American Black Vulture population appear consistent with those described by Reeves.[1] Initial observations of the marks left on the specimens provided to the respective Bald Eagle and Raven populations appear macroscopically to be distinct in overall morphology (i.e., length and overall appearance), from those noted in the Vulture populations, and from those noted in Reeves.[1] Microscopically, however, all marks appeared to display a similar, rounded cross-section very similar to that of the aforementioned populations.

Because the ranges of these birds vary between species, especially due to seasonal migration, analysis of differential taphonomic markings left by avian scavenging will provide valuable information about postmortem interval and location of death (i.e., if the remains are moved postmortem). For example, Bald Eagles are normally only present in the Desert Southwest and the Midwest during the winter (with the exception of a small, year-round population in south-central Arizona).[2] Human skeletal remains found in non-winter months in these geographic regions and displaying marks that are consistent with Bald Eagle scavenging can then be inferred to have been exposed for at least a portion of one winter, further refining postmortem interval and taphonomic processes.[1]

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Avian Scavenging, Postmortem Interval, Taphonomic Processes

H16 Stature Estimation in Modern Thais: Are Population-Specific Estimation Equations Necessary?

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After attending this presentation, attendees will understand why the development and use of separate stature estimation equations for different ancestral groups are necessary in forensic anthropology.

This presentation will impact the forensic science community by demonstrating that the stature estimation accuracy of linear regression equations developed for a given population declines when applied to a different population, in this case the Thai. Additionally, this presentation provides new stature estimation equations developed specifically for modern Thai individuals, a group that until recently has been largely overlooked with respect to stature estimation studies.

Stature estimation is one aspect of a biological profile utilized by forensic anthropologists to assist law enforcement with the identification of unknown human remains. The use of population-specific stature estimation equations by forensic anthropologists increases the precision and accuracy of stature estimations and of identifying an unknown individual who is thought to come from a particular population. The *Daubert* standard requires forensic anthropologists to demonstrate the scientific validity of their methodologies through empirical testing, peer review and publication, and calculating error rates. External validity must be tested and is a requirement when procedures based on one population are applied to another population. Modern Thai males and females exhibit average statures that are considerably smaller than those of American Blacks and Whites, and Hispanic males. Mean stature for Thai males is approximately 165cm, while that of American Black males is 175cm, American White males is 176cm, and Hispanic males is 170cm; Thai females have a mean stature of approximately 155cm, whereas the mean stature for American Black and White females is 164cm.[1] For this reason, the development of population-specific stature estimation linear regression equations appears necessary.

To date, two stature estimation studies have been published for the modern Thai.[2,3] The Mahakkanukrauh study was performed on a sample from Chiang Mai, Thailand, in the northwestern region of the country. The authors noted that their linear regression equations produced unusually high standard errors, particularly with respect to the female equations. The Pureepatpong study was based on a sample population from Bangkok, located in southern Thailand. The linear regressions proposed in the Bangkok study had lower standard errors and higher R^2 values than the Chiang Mai regressions; however, the Bangkok sample population exhibited lower mean statures and limb lengths than both the Chiang Mai sample and the current study's sample population.

The lengths of five long bones (femur, tibia, humerus, radius, and ulna) were collected from 106 modern Thai at the Khon Kaen University Hospital, Thailand. Outliers were removed and linear regression formula and prediction intervals for stature were calculated using *R*.[4] Additionally, regression equations in FORDISC 3.1 based on American White, American Black, and Hispanic populations were applied to the Thai sample.[1] Results showed that the femur is most highly correlated with stature in both males and females, as in other studies, and therefore provides the best single bone stature estimation equations. Compared to the results of the previous Thai stature studies, the results of the current study produced the lowest standard errors and highest R^2 values. 90% prediction intervals at the mean using bicondylar femur length and maximum femur length, respectively, were 160.3cm to 168.9cm for Khon Kaen males and 150.3cm to 161.1cm for Khon Kaen females.

Upon comparison, estimation of Thai stature using the FORDISC 3.1 linear regressions resulted in lower R^2 values, higher standard errors, and wider prediction intervals across the board. Black equations for males and females underestimated Thai stature overall, whereas the White and Hispanic equations tended to overestimate Thai stature. Larger prediction intervals sacrifice precision for greater accuracy; however, in a forensic context, accurate but imprecise stature estimations are not as useful to law enforcement officials. These findings indicate that although stature prediction equations that were intended for use on a different population will work, these estimates will contain greater error, especially when moving farther away from the sample mean.

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Stature Estimation, Linear Regression, Southeast Asians

H17 Online Pedagogical Methods in Forensic Anthropology: Effective Strategies for the Virtual Field and Lab

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After attending this presentation, attendees will become aware of online or e-learning alternatives for developing and disseminating curricula in introductory forensic anthropology courses. This

presentation will offer an example of an online forensic anthropology course that meets objectives as equivalently and effectively online as it does face-to-face.

This presentation will impact the forensic science community by increasing awareness of online learning and its application to introductory forensic anthropology courses. Online learning (for example, fully online courses, blended learning, game-based learning, and social networking) is one of the fastest growing trends in education. The advantages of online learning to students, instructors, and universities are well documented with the exception of certain disciplines like forensic anthropology. While there are many empirical studies of online learning, there is a paucity of research on best practices in anthropology, especially subdisciplines in bioanthropology, archaeology, and forensic anthropology. It would be useful to know how anthropologists are utilizing online learning tools and carefully consider the innovations that will increase the quality of online anthropology courses.

The concern that courses cannot be taught effectively online seems to be elevated in certain disciplines where the lack of hands-on laboratory demonstrations and exercises presents a major disadvantage. Forensic anthropology laboratory and field experiences are outstanding aids to learning but even traditional classroom environments may not have osteological collections or opportunities for conducting work in the field due to financial or logistical constraints. Indeed, online courses in forensic anthropology may require more creativity and effort than other anthropology courses but there are effective mechanisms to conduct virtual laboratory sessions without the tactile use of human remains.

The effectiveness of the online approach was tested by comparison of student learning outcomes and satisfaction between online and face-to-face forensic anthropology courses taught at Oregon State University and Western Oregon University. All sections of course material (readings, lectures, assignments, due dates, and other requirements) were similar in content but administered differently for each course. Four indicators of student success were examined using independent *t*-tests: cumulative lab scores, cumulative quiz scores, project scores, and total points earned. Two indicators of student satisfaction were examined, also using independent *t*-tests: satisfaction with the course as a whole and the instructor's contribution to the course. These two components of the evaluations were the same in content but administered differently between online and face-to-face courses.

Results demonstrate that course objectives are equally achieved and teaching is effective in both cases as student success is as high online as it is on campus. The results of the *t*-tests showed that of the four comparisons, none were significantly different (*p-value*>0.05). Additionally, student perceptions of the courses are above average for both methods of delivery. The results of the *t*-tests showed that there was no statistically significant difference in combined course and instructor satisfaction. Unedited student comments also tend to demonstrate fulfillment with the online course.

This study is likely the first to examine the efficacy of online instruction in forensic anthropology. The results provide a clear example of the design and delivery of online labs and experimental projects that may be useful for other anthropology and forensic science courses. Despite the challenges associated with using skeletal remains as teaching tools, it appears that students succeed in learning online and are satisfied with the results. Technology provides methods that meet today's student, instructor, and institutional needs but additional discussion is needed to improve online learning within forensic anthropology.

Forensic Anthropology, Pedagogy, Online Learning

H18 Positive Identification Using Chest Radiographs: Standards for Minimum Number of Concordant Points

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After attending this presentation, attendees will gain a better understanding of: (1) a method of decedent identification via comparison of vertebral column characteristics between antemortem and postmortem radiographs; (2) the associated statistical probabilities of a positive identification using this method; and, (3) the variance of those probabilities based on the quality and several other characteristics of the specimens and radiographs.

This presentation will impact the forensic science community by serving as a statistically valid and systematic means for positive identification of decedents when more traditional methods of identification are not available, or when those traditional methods need to be supplemented.

Positive identification is of primary importance for case resolution and bringing closure to the victims' families. Difficulties surrounding the identification process can result from taphonomic processes that result in incomplete recovery of skeletal materials and/or from inadequate antemortem records. Therefore, a variety of identification methods that utilize various anatomical structures is essential for timely and accurate identification. While there are a number of methods used to make positive identifications through radiographic comparison, many lack the scientific rigor necessary to make them admissible in court. The utility of radiographs for positive identification was examined in accordance with the United States Federal Court ruling (*Daubert vs. Merrell Dow Pharmaceuticals*, 509 US.579, 1993) and National Academy of Sciences (NAS) 2009 Report, *Strengthening Forensic Science in the United States: A Path Forward*, which calls for more testable and reliable scientific research. To date, there has been ample research exploring morphological variation in the frontal sinus, chest, and vertebrae for positive identifications; however, much of this research has focused on the investigator assessing morphological similarities or dissimilarities via side-by-side comparisons, which would not satisfy the *Daubert* criteria or NAS report. The utility of radiographs for medicolegal purposes is shown by the uniqueness of certain features of the skeleton in previous research; however, there is a need to quantify their uniqueness. Although vertebral radiographs are commonly used in the identification process, standards do not currently exist regarding a minimum number of concordant points that should be used. The case reports in the forensic literature show that one to four points with no discrepancies have been used to determine identity.

The purpose of this pilot study was to examine the vertebral column in a known sample of 60 antemortem and postmortem chest and abdominal radiographs from the North Carolina Office of the Chief Medical Examiner to: (1) evaluate the uniqueness of traits observed in the vertebrae; and, (2) explore the minimum number of corresponding traits necessary to make a positive identification in order to address the issue of probabilities, a growing concern in the court systems. Thirty comparisons were antemortem and postmortem radiographs from known individuals. To represent the unknown or no-match sample: either an antemortem or postmortem radiograph was compared to a randomly selected individual. The following eight traits were scored for each radiograph: cervical morphology (e.g., pedicle, spinous process shape, etc.), thoracic morphology, lumbar morphology, quality of antemortem X-ray (e.g., good, average, and poor), quality of postmortem radiograph, presence of congenital anomalies, elapsed time between antemortem and postmortem radiographs, and condition of remains (e.g., skeletonized, decomposed, etc.).

To explore patterns and relationships of the data, a robust data mining technique called a classification decision tree was applied. For categorical data, a G^2 or the likelihood-ratio chi-square for the variables is computed and used for multi-level split of the data. The advantages of decision trees are that they are easy to interpret, they are able to

handle both categorical and numerical data, and they are robust. Results show that the anatomical elements with the most predictive value were the lumbar vertebrae and indicate that if you have more than five concordant points, you have a 71% probability of correct classification (Fig. 1). If the postmortem X-rays are good or average quality, then the probability increases to 90% and if the antemortem radiographs are average quality, the probability of correctly matching the radiographs increases to 93%. Likewise, if there are less than five points of concordance, there is only a 33% probability of correct classification and if the antemortem radiographs are good or average quality, then the probability increases to 62%. Thus, these results show that a minimum of five points of concordance are needed with good or average quality postmortem radiographs in order to have a 90% probability of correctly classifying or making a positive identification based on the lumbar vertebrae. The cervical and thorathic vertebrae were not found to be unique enough to be used to make a positive identification on their own. However, the use of cervical and thorathic vertebrae in a larger sample is currently being examined.

This project was sponsored by the National Institute of Justice (2010-DN-BX-K214).

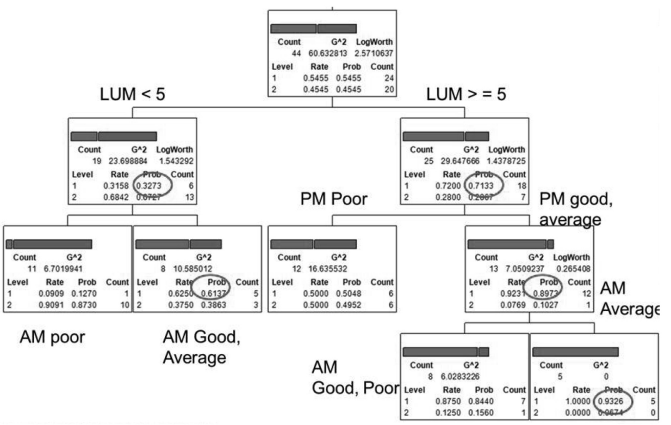

Fig. 1. Results showing the lumbar vertebrae as having the most predictive power and probabilities associated with corresponding traits.

Radiographs, Positive Identification, Number of Traits

H19 Adipose Distribution as a Predictive Model for Scavenging Sequencing and Intensity

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The goals of this presentation are to: (1) provide an assessment of current scavenging models; (2) provide an evaluation of actualistic experimental data; and, 3) provide a discussion of the role of adipose in scavenging sequencing and intensity. Attendees will be provided with examples of scavenging patterns from actualistic experiments using pig and deer carcasses and from forensic case report documentation of curated human remains housed at the California State University, Chico Human Identification Lab (CSUC-HIL).

This presentation will impact the forensic science community by providing a critical evaluation of current scavenging models and discussing the role of adipose in scavenging behavior.

Current anthropological scavenging theory states that elements disarticulated the earliest should show the greatest amount of damage. Much of the literature also states that the ventral thorax should be one of the first areas of the body to be scavenged. This study, however, shows that sometimes the internal organs are the last portions scavenged.

The actualistic study associated with this presentation was conducted in October 2009 and November through December of 2011 at the Big Chico Creek Ecological Reserve (BCCER) in Butte County, California. A single adult mule deer and five 100-pound pig carcasses were placed at six different locations within the BCCER. Digital game cameras were positioned at each of the sites in order to monitor scavenger activity. Two pictures were taken each time the motion-sensitive laser was triggered, with a delay of one minute between each image. Sites were monitored daily and the camera was repositioned if the carcass had been moved out of the camera's field of view. The carcasses were placed on the surface, and to prevent immediate removal from the site location, each carcass was tied down to rebar stakes with lengths of wire wrapped around the forelimbs and hind limbs. Documented scavengers included: black bear, gray fox, turkey vulture, red-tailed hawk, golden eagle, and the common raven. From the camera documentation, the sequence of tissue consumption and disarticulation were recorded and analyzed. The scavenging damage was then compared to 21 forensic cases curated at the CSUC-HIL. The deer, pig, and human skeleton element representation, and inter- and intra-element scavenging patterns, were documented in detail.

Results indicate that the carcasses were consumed in a predictable sequence from areas of high body-fat content to areas with low fat content. In the deer carcass, scavenging damage occurred first to the thorax and anal region, with all internal organs removed and consumed in the first 24 hours. The deer was completely skeletonized, disarticulated, and scattered by the second day. Total body fat of an adult female mule deer ranges from 3.7 to 10.9 percent (Torbit et al. 1988).[1] Conversely, in the case of the pigs, the skin and subcutaneous tissue were consumed first, followed by the forequarter, hindquarter, and lastly, the internal organs. In modern, commercially produced pigs, total carcass fat is kept at approximately 20 percent; this percentage is similar to the average American adult (Kouba and Sellier 2011, MedlinePlus 2011).[2,3]

The pattern documented in the pig carcasses, with regards to scavenging intensity and adipose amounts, was mirrored in the human skeletal sample. The most heavily scavenged portions of the human skeletons were the innominate, proximal ulna, proximal tibia, and proximal humerus; these areas are all surrounded by rich fat deposits. The higher rates of damage in high-adipose areas may occur because of the higher caloric, and thus energetic, return of fat-rich areas compared to protein- or carbohydrate-rich areas. Thus, areas that are richer in fat should have a higher degree of scavenging intensity than areas lower in fat.

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Taphonomy, Scavenging, Adipose

H20 The Use of Joint Surface Pathology to Reassociate Commingled Human Remains in Forensic Anthropology

Colleen Cheverko, BS, 635 West 6th Ave, Apt 9, Chico, CA 95926*

After attending this presentation, attendees will have a better understanding of how joint surface pathology can be used to reassociate commingled human skeletal remains.

This presentation will impact the forensic science community by providing forensic anthropologists who are often confronted with the challenges of reassociating commingled skeletal remains an additional sorting tool.

Osteometric sorting, articulation, pair matching, taphonomic similarities, and DNA sequencing are among the established techniques used to reassociate commingled skeletal remains. The technique of articulation is based on the principle that two bones in articulation form a congruent joint; however, the effects of joint surface pathologies such

as osteoarthritis on articulation are unknown. These pathologies often exclude the use of traditional methods, warranting the development of new techniques to reassociate affected commingled remains.

The goal of the study was to evaluate the expression of osteoarthritis as a possible tool for sorting commingled remains. Medical literature indicates that the expression of osteoarthritis is consistent within an individual across joint surfaces in autopsy and surgical patients; however, bioarchaeological literature shows the variation in expression of the pathology is dependent on the method used to identify it.[1] To insure consistency in scoring osteoarthritis, the authors used the criteria published by Buikstra and Ubelaker to determine presence of osteoarthritis and the criteria established by Jurmain to determine the severity of the joint disorder.[2,3]

For this study, 258 discrete individuals from the Phoebe A. Hearst Museum of Anthropology were examined. The individuals included in the study were aged 30 years or older and had 50 percent of two or more surfaces from the same joint. The degree of lipping, eburnation, and porosity was examined in each synovial joint of the skeleton and a severity score was assigned that described the joint degeneration.

Overall, the majority of the osteoarthritis was expressed as marginal lipping and porosity; therefore, many of the joint surfaces were scored with a severity score of one. Chi-square tests were used to analyze the frequency of osteoarthritis in the hip, knee, elbow, and shoulder. The results showed that presence of osteoarthritis was consistent across surfaces of a joint. For example, the radial head was not marked with osteoarthritic changes if the capitulum of the humerus was not. Similarly, the medial condyle of the tibia was likely scored as one if the medial condyle of the femur was scored as one.

The study showed that osteoarthritis is a useful tool to reassociate commingled skeletal remains; although, it is most useful when used in conjunction with other techniques. While pathology of the joint surface may preclude the use of other methods such as the measurement of joint surfaces, joint pathology is a valid criterion to reassociate discrete individuals. This study only examined osteoarthritis; future studies should evaluate other pathological conditions.

Despite a great deal of past research, there is a need to develop additional tools to facilitate reassociation of discrete individuals. Future studies should investigate joint surface pathologies from modern and archaeological collections to increase our understanding of these pathologies and their value for reassociating commingled remains.

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Commingling, Mass Burials, Joint Pathology

H21 The Power of Contextual Effects: A Study of Biasability in Visual Interpretations of Trauma Analysis on Skeletal Remains

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After attending this presentation, attendees will understand how contextual information can bias assessment of trauma images.

This presentation will impact the forensic science community by demonstrating how bias can impact forensic anthropology and how contextual information can affect objective assessments by scientists with a range of experience and ability.

The potential for contextual information to bias assessments in the forensic sciences has been demonstrated, focusing on the DNA, ballistics, and friction ridge analysis disciplines. This has been discussed in the National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward.* However, in many forensic disciplines, such as anthropology, the presence of bias, its impact on objectivity, and how to mitigate its effects is still not fully assessed or appreciated. Effects that may impact the judgment and decision-making of forensic anthropologists need to be measured. No studies have been performed within the discipline assessing possible biasing effects within visual analysis.

Biasability potential within forensic anthropology was examined by constructing an experiment that analyzed the effects of external manipulations on judgment and decision-making in visual trauma assessment. Three separate websites were created containing 14 identical images of skeletal remains presenting a range of trauma. Each website presented participants with different contextual information. The three separate contexts described human rights mass grave excavations, a 19th-century archaeological excavation setting, and a control scenario with no specific contextual information provided. Ninety-nine participants were equally distributed and randomly assigned to one of the three scenarios. Participants completed a survey noting qualifications and experience, and were asked to assess the presence of trauma in the images and to describe their confidence in their interpretation by scoring for level of certainty. The interpretation of presence of trauma was assessed to determine if it would differ for the same images across the different scenarios.

The results indicated a bias correlation between the three scenarios, indicating a higher likelihood of identifying trauma within the mass grave excavation context. A significant biasing effect was associated with four of the images, notable for their ambiguous and distinct nature. Participants with less experience were more likely to interpret the presence of trauma. This research demonstrates that bias can be detected in the field of forensic anthropology, highlighting the importance of recognizing issues that may influence interpretation during investigation and analysis, as well as the need for further research on how to mitigate these effects.

Anthropology, Bias, Trauma Assessment

H22 Did I Teach Them That? Setting and Assessing Goals for Student Learning for an Introductory Forensic Anthropology Course

Phoebe R. Stubblefield, PhD, Univ of North Dakota, Dept of Anthropology, 236 Centennial Drive Stop 8374, Grand Forks, ND 58202*

After attending this presentation, attendees will be able to describe an appropriate goal for an introductory forensic anthropology course as well as the format of the assessment tool used in this presentation.

This presentation will impact the forensic science community by disseminating information regarding a technique for determining the effectiveness of forensic anthropology (or other science) courses.

In this presentation, a technique used for several years for assessing student learning in an introductory forensic anthropology course is described. This technique begins with a statement of the goals of the course, followed by design and implementation of a tool for determining if particular goals have been met. After viewing this presentation, observers should be able to describe an appropriate goal for an introductory forensic anthropology course and describe the format of the assessment tool used in this presentation. This presentation will have an impact on educators in the forensic science community by disseminating a technique for determining the effectiveness of forensic anthropology (or other science) courses.

Instruction of the course "Introduction to Forensic Anthropology" at the University of North Dakota is subject to learning goals occurring at the instructor, department, and university levels. Statement of the goals establishes the frame of reference for developing the assessment tool. Instructor level goals are public and private. Private goal setting is how instructors make clear to himself or herself the intent and significance of the course, and will affect the choice of course design (e.g., lecture, lecture with lab or hands-on activities, emphasis on videos, emphasis on small group activities), texts used, and frequency of offering. Institutionally based limits on course design should be reflected in departmental or institutional goals for the course. The instructor may set public goals, stated on the syllabus, based on criteria such as his or her own interest in what students should derive from the course, the content of the texts used in class, and/or requirements set by the department and institution. For example, a personal interest-derived goal for Introduction to Forensic Anthropology may be "Name and describe the contributions of famous forensic anthropologists." An institutional (department level) goal is "to demonstrate knowledge and understanding of the sources of biological and cultural variation and how these change over time." If a large number of public goals are applied, an overall assessment program may be needed to support inspection of different goals in different academic years, lest the assessment process become cumbersome.

The tool used to assess student learning is a test composed of Likert scale and short answer questions administered at the beginning and end of the course. The questions are designed to provide indirect (from the Likert questions) and direct (from the short answer questions) assessment data of the public goals for the course. For the tool to be relevant, the questions must be derived from the course goals and, as a consequence, may vary somewhat from year to year and are subject to revision. The Likert questions in this tool tend to demonstrate student confidence in knowledge. This is an indirect measure of student learning as it reflects only the student's opinion. The question, "I have a basic understanding of human skeletal anatomy, enough to name major bones," is answered with a spectrum of "describes me" responses. An appropriate short answer question provides data for the direct assessment, in this case being "Name all the long bones of your upper limb." Students may claim knowledge in the Likert, but then that knowledge must be demonstrated in the short answer. Here the instructor interprets the short answer response to determine if students can demonstrate the knowledge.

Once the tool is created, the greatest work in this process is in collating the data, but this is an appropriate project for a student worker sworn into Family Educational Rights and Privacy Act (FERPA) compliance. Likert questions can be collated in a spreadsheet program and meaningfully portrayed graphically. However, the short answer questions must be coded to allow the student worker to collate the data, which means the instructor must survey the responses and apply codes. Interpretation of the direct assessment data can be perfunctory to address student acquisition of knowledge (typically satisfying institutional goals) or rigorous (typically addressing private goals). In the long bone question, variation in the responses (such as inclusion of carpals and phalanges) may demonstrate that the test question should be reworded or vocabulary clarified in class. An assortment of course goal-Likert-short answer trios, and before-and-after results is presented in order to demonstrate the utility and format of the assessment tool.

Forensic Anthropology, Education, Assessment

H23 Analytical Test Method Selection and Validation of Laboratory-Based Methods

Paul D. Emanovsky, PhD, JPAC-CIL, 310 Worchester Ave, Joint Base Pearl Harbor-Hickam, HI 96853*

After attending this presentation, attendees will have a better understanding of the principles of selecting a method for inclusion in their laboratory's standard operating procedures (SOP) and the necessary steps to validate a laboratory-based method, which may include modifications of longstanding methods.

This presentation will impact the forensic science community by illustrating the principle, spirit, and intent of method validation through the use of a case study-validation of the Hefner Optimized Summed Scoring Attributes (OSSA) method for ancestry determination.

Forensic anthropology laboratories must weigh numerous factors when determining which analytical test methods to include in their SOPs. What is generally accepted as a valid method (*sensu Frye*) by the expert community influences the decision. Further, appropriate weight must be given to adherence to the principles set forth by the *Daubert*, and later, the *Kumo* rulings, which inform the forensic science community on what is admissible to the courts as expert witness testimony and within forensic reports. These rulings stipulate the need for an assessment of a method's relevance, reliability, and, in the case of *Daubert,* the "scientific knowledge" base from which it is developed. Legal requirements, as well as the sentiments developed out of these rulings, certainly have influenced how accrediting bodies recommend best practice and the perceived requirements for minimum standards of a test method. However, the expert community must still vet new and old methods alike to ensure they adhere to these guiding principles. Perhaps more importantly, these methods must also evolve alongside our understanding of the "scientific knowledge" base from which the tests derive *and* the policy-oriented realm in which they will be presented.

Under ISO 17025, the standard used by a testing laboratory to select an analytical method must include considerations of such issues as the needs of the customer, and whether the test is "appropriate." Preferred methods are those published in international, regional, or national standards. Since there are no national (or otherwise) standards in place for forensic anthropological tests, we must move to other tier-utilizing tests published by technical organizations. Also, analytical methods published in relevant scientific texts or journals, or those based on manufacturer's specifications (e.g., scanning electron microscope analysis) serve as *de facto* standards.

Forensic anthropology as a field has certainly taken up the call to ensure the methods used in day-to-day casework meet a *Daubert* challenge. A plethora of validation studies and subsequent modifications to longstanding methods have been presented. Still, not all methods that are published in scientific journals are created equal, and just because a method is published does not make it reliable, valid, or appropriate for the problem at hand. Thus, an additional source for methods which may be selected for inclusion in an SOP is laboratory-developed or adopted methods. This source is acceptable provided the method is appropriate and validated. The validation need only be as extensive as necessary to confirm the procedure fits its intended use; which is a point that could lead to rigorous debate. Confirmation comes from determining the performance characteristics of the intended method.

The Hefner OSSA method was initially developed as part of a doctoral dissertation born from the statistical quantification of the long-used morphoscopic gestalt. The method was then qualified as a laboratory-developed method. As such, it was subject to a planned validation study prior to being accepted into an SOP. Validation could have taken several forms, as will be discussed; however, ultimately the method was tested on an independent sample of American White (n=79) and American Black (n=49) individuals from the William M. Bass Donated Skeletal Collection. After applying the OSSA method, which requires scoring six cranial nonmetric traits according to published protocols, a systematic assessment of the uncertainty involved in the test results was undertaken. Based on the validation study results,

performance indicators point to the efficacy of the Hefner OSSA method (sensitivity=87.76%, specificity=89.87%, correct classification rate=89.06%, error rate=10.94%, positive predictive value=84.31%, and negative predictive value=92.26%; in this example, the positive predictive value (true positive/(true positive + false positive)) is the probability the individual is "Black" when OSSA indicates "Black"). The validation of OSSA is ideal to illuminate a discussion of the principle, spirit, and intent of method validation and selection.

Method Validation, Accreditation, Ancestry Determination

H24 Resolution of Cold Cases: A Multidisciplinary Approach to Identifying Remains Previously Interred as Unknown

Debra P. Zinni, PhD, JPAC-CIL, 310 Worchester Ave, Bldg 45, Joint Base Pearl Harbor-Hickam, HI 96853*

After attending this presentation, attendees will gain strategies for using a multidisciplinary approach to resolve cold cases.

This presentation will impact the forensic science community by providing knowledge and methods for employing a multidisciplinary approach to resolve cold cases, particularly when DNA analysis is not available.

The Joint POW/MIA Accounting Command Central Identification Laboratory (JPAC-CIL) is charged with conducting global search, recovery, and laboratory operations to identify unaccounted-for Americans from past conflicts in order to support the Department of Defense's personnel accounting efforts. During the 1950s, the remains of deceased service members from the Korean War were processed through the Central Identification Unit (CIU), Kokura, Japan, in order to establish identity; however, 867 sets of remains were determined to be unidentifiable and were buried as "Unknown" with full military honors at the National Memorial Cemetery of the Pacific (NMCP) in Hawaii. In preparation for interment, the remains were subjected to postmortem chemical processing that, in turn, has affected DNA recovery. As part of the JPAC mission, CIL historians and analysts review case files (originally generated by the CIU) for each set of remains interred as "Unknown" to determine cases where new technology or information produces a high likelihood of identification. The CIL has yielded a high success rate in identifying unknown remains from the Korean War, despite problems with DNA analysis. This is accomplished by employing a multidisciplinary approach, utilizing military historians, anthropologists, and odontologists during the research and analytical phases. Analysts associate individuals with unknown remains through a combination of extensive archival research into loss locations and dates, recovery locations and dates, POW movements, and review and re-analysis of the anthropological and odontological information present in the files. Following exhumation of the remains, laboratory scientists conduct anthropological and odontological analyses. Since DNA analysis cannot currently be utilized for these cases, the CIL relies on comparison of the circumstantial information, biological profile, dental information, chest radiographs, and photographic superimposition to support a positive identification for these remains.

To date, 34 unknown graves from the Korean War have been exhumed from the NMCP and re-analyzed at the CIL by analysts "blind" to information on these cases. These analyses have resulted in the sorting of 37 individuals, of which 24 have been positively identified. Post-identification, the biological profile generated by the staff at the CIU in the 1950s, was compared to the biological profile produced by the CIL anthropologists (current analysis) in order to refine the methodology of associating individuals with unknown remains. In addition, both biological profiles were compared to the actual biological profile of the identified individual. From these analyses, several trends have appeared in the data: the CIU age ranges only captured the actual age of the individual 50% of the time. In regard to the ages that were not captured, 75% were overestimated in age. The CIL age ranges, in comparison, captured the actual age 96% of the time. The CIL age ranges were, on average, wider than the CIU age ranges, which may account for some of the error. Stature estimates produced at the CIU yielded a 26% accuracy, with an almost even distribution of cases being overestimated (47%) and underestimated (53%) in stature; whereas the CIL prediction interval for stature captured the actual stature in 96% of the cases. The low percentage generated by the CIU stems from narrow prediction intervals, which in some cases were point estimates. Race was correctly assessed in 87.5% of the CIU case notes and in 100% of the CIL forensic anthropology reports.

The data collected from the cases of unknown individuals who are now identified aids in the refinement of analyses, archival research program, and efforts of associating unaccounted-for individuals with remains that are buried as unknown. Continued development of background research and advances in forensic science increase the ability to identify remains previously interred as unknown.

Cold Case, Identification, JPAC-CIL

H25 Results Based Management and Forensic Anthropology: The Ontario Experience—Part 1: Human vs. Non-Human

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After attending this presentation, attendees will understand how to apply Results Based Management (RBM) models to their own areas of practice, and how to assess the impact of a new input (in this case specifically, a full-time forensic anthropologist).

This presentation will impact the forensic science community and, specifically, jurisdictions utilizing forensic anthropology by providing assessment tools for their particular area of forensic science in order to demonstrate a measurable and positive impact of the use of the science in a language that governments understand (fiscal responsibility, accountability, stakeholder satisfaction, etc.)

Forensic anthropologists provide services to medicolegal death investigation systems. These systems are usually government-based, and are part of a broader legal system. Death investigations can also inform other government agencies, such as public policy and health departments/ministries among others. RBM strategies or approaches are those that focus on achieving outcomes and impact, rather than activities and input. RBM has been widely embraced by international development agencies as a way to assess the effectiveness of the billions of dollars of aid funneled through the United Nations. The end result of RBM is to improve the performance of an organization as well as its accountability. While all forensic anthropologists and the organizations which utilize their services or employ them understand why they are needed, the impact of the use of forensic anthropology is not often measured on a broader scale which may include such things as risk and economics.

This presentation introduces the first of a three-part analysis of the outcomes and impact of having a full-time forensic anthropologist in a government death investigation service. Here, the assessment of bones for their origin (human vs. non-human) over a two-year time period (July 2010 to July 2012) is presented. In Ontario, the forensic anthropologist works in a medical coroners' death investigation system, legislated by The Coroner's Act R.S.O. 1990. The Act outlines the duties of both coroners and forensic pathologists in the system. The responsibility of the coroners begins when they are informed about a body of a dead person in their jurisdiction. Although the remains of animals are not persons, historically, these have been assessed by coroners and/or pathologists. In the recent past, animal bones were also assessed by fee-for-service forensic anthropologists. It is known (anecdotally) but not documented that these remains are also assessed by police, academics, veterinarians, and local doctors. In July 2011, a salaried, full-time forensic anthropologist began working in Ontario.

In the period assessed, approximately 400 cases of non-human remains were reported on (ca. 200 per year). In the year following the hiring of a full-time forensic anthropologist, approximately half of these remains were assessed via digital images sent to the forensic

anthropologist from the scene where they were found (in a few cases, they had been moved to a police station). The other half were assessed either at a scene, a police station, or a forensic pathology unit by a coroner or forensic pathologist travelling to the scene to look at the bones. Utilizing the RBM model, the differences between the new approach (forensic anthropologist and digital images) and the historical approach were analyzed. The outcome effects were assessed by a number of factors including fiscal/economic (costs associated with paying fee for service coroners, police-person hours spent holding scenes or photographing remains, case management and reporting costs, etc.), stakeholder satisfaction, and risk, among others. A positive impact can already be demonstrated by the use of the forensic anthropologist and digital identifications at many levels; however, to date there is no widespread systemic change, and some reasons for this will be presented. RBM is a useful model which provides substantive data for other jurisdictions which may be considering the addition of a full-time specialist in a forensic field that may currently be serviced by fee-for-service specialists. Future analyses will look at the impact of full-time forensic anthropologists with other types of cases, including those that are not of recent forensic interest and those that are.

Forensic Anthropology, RBM, Impact

H26 The Pima County Office of the Medical Examiner Forensic Anthropology Postdoctoral Fellowship: An Advanced Training Model for Newly Emerging Forensic Anthropologists

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After attending this presentation, attendees will learn about the one-year forensic anthropology postdoctoral fellowship now being offered at the Pima County Office of the Medical Examiner (PCOME) in Tucson, Arizona.

This presentation will impact the forensic science community by providing an example of one of the two forensic anthropology fellowship training programs currently being administered by a Diplomate of the American Board of Forensic Anthropology (D-ABFA) and working within the medical examiner/coroner system in the United States.[1]

As a fast-paced medical examiner's office with an elevated number of unidentified human remains, the PCOME offers an exceptional training opportunity for forensic anthropology PhDs hoping to gain practical experience within the medical examiner system. With nearly 200 anthropology consults requested each year, the PCOME exposes the postdoctoral fellow to a substantial number of cases and a variety of anthropological case types. Moreover, desert conditions, the vast number of Southwest Hispanic undocumented migrants dying within PCOME jurisdiction, and the large number of unidentified human remains cases presents a rather unique caseload. Within an average year at the PCOME, skeletonized remains cases make up a considerable percentage of anthropological consults, and thus expose the postdoctoral fellow to one of the largest caseloads of skeletonized remains within the medicolegal system. In addition, a substantial diversity in anthropological consults are requested for non-human remains, as well as for fresh, decomposed, burned, and fragmentary human remains for local residents and undocumented migrants dying within PCOME's jurisdictions. Because the PCOME performs medicolegal investigations for 11 of the 15 counties in Arizona, diversity in geographical factors, such as climate and elevation, allows the postdoctoral fellow to appreciate differences between the Sonoran Desert and mountainous regions in estimating the postmortem interval. The PCOME also recognizes its role as a training institution because of the substantial casework and is in full agreement with the Scientific Working Group in Forensic Anthropology (SWGANTH) that this casework should be utilized to offer additional training to emerging forensic anthropologists.

At the PCOME, the forensic anthropology postdoctoral fellow functions as one of two fulltime forensic anthropologists and is responsible for a majority of the anthropological exams requested by one of the six forensic pathologists in the office. Anthropology cases at the PCOME cover a wide range of responsibilities and any consultation may include: scene recovery, determination of the biological profile and postmortem interval, dental examination, assessment of skeletal trauma, sampling of skeletal tissues for DNA analysis, and positive identification through comparative medical or dental radiography. The fellow is independently responsible for all processing and analysis of casework, authors his/her own case reports in accordance with SWGANTH and ABFA recommendations, and may be called to testify as an expert witness if any cases go to trial. The PCOME believes this level of autonomy is essential in rounding out the fellow's skill set. In addition to forensic examinations, anthropologists at PCOME are responsible for entering unidentified remains cases into the National Missing and Unidentified Persons System (NamUs); as well as comparing the biological profile and dental information of probable undocumented migrants with a list of missing persons reports compiled by foreign consulates and non-governmental organizations. Once an unidentified body is released, the anthropologists still maintain responsibility and continue to follow up on missing person's reports and NamUs comparisons. Aside from anthropological consultations, the postdoctoral fellow may also be involved in a number of other activities, including scientific research, presentations or training within the medicolegal or local university community, and the supervision and mentorship of both undergraduate and graduate student anthropology interns.

The PCOME forensic anthropology postdoctoral fellowship provides another potential model for an advanced training program that prepares the emerging forensic anthropologist for a promising future in the field. Through the fellowship, the postdoctoral fellow gains an immeasurable amount of forensic anthropological case experience and develops leadership skills and confidence in his/her ability to perform a wide range of anthropological consultations. Furthermore, the fellowship exposes the newly emerging scientist to the role of forensic anthropology within a large, high-functioning medical examiner's office and teaches how to directly cooperate with law enforcement, death investigators, and forensic pathologists. Most importantly, the PCOME postdoctoral fellowship allows the newly emerging scientist to work directly with a board-certified forensic anthropologist while attaining postdoctoral experience that will allow for further preparation for the job market and ABFA certification.

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Forensic Anthropology, Postdoctoral Fellow, Medical Examiner

H27 The Contributions of Richard Jantz to the Development, Implementation, and Continuance of the Forensic Anthropology Data Bank

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After attending this presentation, attendees will have a better understanding of the historical development and current status of the Forensic Anthropology Data Bank (FDB).

This presentation will impact the forensic science community by detailing the significance of Richard Jantz's involvement in the implementation and continuation of the FDB.

The FDB was created in 1986 with a grant from the National Institute of Justice (NIJ). The concept of this NIJ-funded research initiative was sparked by the need to obtain modern skeletal reference collections: it was clear that the Terry and Hamann-Todd collections did not best represent variation in modern Americans.[1] In effect, Dr. Jantz recognized the issue of external validity that would be stressed decades later in the *Daubert* guidelines. Clyde Snow first commented on the need for modern skeletal reference data and suggested data bank curation as a solution.[2] In 1983, a committee appointed by then American Academy of Forensic Sciences (AAFS) Physical Anthropology Section President Michael Finnegan further developed the concept. The initial committee consisted of Clyde Snow, Larry Angel, Stanley Rhine, and Richard Jantz, with Douglas Ubelaker replacing Clyde Snow at a later date.[3] Ellis Kerley also played a critical role in the development of the FDB, providing external reviewer support.[4] While these historical aspects of the development of the FDB are well described in the literature and by the professionals that observed them directly or indirectly, Richard Jantz's impact on the implementation and continuation of the FDB is, to an extent, lesser known.

Originally designed to meet the need for archiving metric, non-metric, and demographic information from positively identified forensic anthropological cases that are returned to family, the FDB provided the first relational database of modern skeletal data made publicly available to further research in forensic anthropology. The FDB is unique as it is derived from modern forensic cases and provides up-to-date, timely reference data that meet the needs of the changing demographic structure of our society. The FDB was initially conceived as a tool for case submissions by practicing forensic anthropologists through standard data collection procedures, archiving this data to further the discipline. However, as pointed out by Jantz and Moore-Jansen, a perceived problem of the FDB was how much time forensic anthropologists can justifiably spend on data collection efforts, considering other job commitments, time spent processing the remains, and compiling information from the medical examiner, medical records, law enforcement, or family.[2]

To circumvent the time constraints faced by these practitioners, Jantz or his graduate students visited numerous collections, measuring and recording biological information on a large number of skeletons. To date, Richard Jantz is directly responsible for collecting over 70% of the data in the FDB. Dr. Jantz has also been instrumental in diversifying the FDB data. Through working with collaborators from outside institutions, a large number of otherwise under-represented groups, such as American Blacks, Hispanics, and Japanese, are now thoroughly represented, necessary for updating forensic anthropological methods and furthering relevant research. Funding for travel to various collections (following NIJ grant funding) has been supported through an FDB assistantship provided by the Department of Anthropology at the University of Tennessee and FORDISC sales. Numerous publications, theses, and dissertations have benefited from data obtained from the FDB. The majority of forensic reference samples in FORDISC come directly from the FDB. Without Dr. Jantz's dedication to keeping up-to-date samples in the FDB, there would not be the relevant resources needed to further methodological development within our discipline.

Although Richard Jantz has retired, he still continues work on upkeep and expansion of the FDB. The FDB will continue, and the types of data contained will also be expanded (including radiographs and CT scans). The value of the data bank concept was so well illustrated by Dr. Jantz's work and the *Daubert* ruling that it will continue to be an essential source on modern human variation. A detailed report of Dr. Jantz's specific contributions to the continuance of the FDB along with those that have supported his endeavors will be discussed.

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Richard Jantz, FDB, Anthropology

H28 Introducing FOROST: An International Free-Access Visual Forensic Osteology and Osteopathology Metabase

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After attending this presentation, attendees will learn about a ***For***ensic ***Ost***eological (FOROST) (www.forost.org), image database of osseous trauma and pathologies.

This presentation will impact the forensic science community by presenting an educational tool that will assist forensic anthropologists with interpretations of trauma and pathologies.

DNA has revolutionized the way forensic scientists determine sex and identity, but detecting method, manner, and cause of death from bones remains a largely observational endeavor. Interpretation of bone modification and pathology is a visual and experiential skill, relying heavily on the background of the forensic osteologist. Each case is different, and more examples from which to draw comparisons mean better-equipped osteologists. Traditionally, such background is built first through books and "hands on" experience in a single collection, then gradually augmented through contact with multiple collections and cases. The FOROST initiative adds to this set of background material a large-scale comparative database of skeletal lesions that can be accessed from anywhere in the world.

The practice of electronically serving visual osteological information is nearly as old as the public Internet itself. Phil Walker and Ed Hagen developed the first professionally viable osteology web interface, a human dentition visualizer, at the University of California, Santa Barbara, in 1991 and 1992.[1] As digital cameras became viable in the late 1990s, the capacity for the Internet to open remote osteology collections to distant researchers was recognized, and a publication outlining the basic framework for a public visual osteology database was one of the outcomes of a 2001 collaboration between the Human Evolution Research Center, University of California, Berkeley (HERC-UCB) and Universidad Nacional Autonoma de México (UNAM).[2] HERC-UCB continued development of data systems for public databases of fossils and hominid casts.[3-7] Reinitiated collaboration between HERC-UCB, UNAM, and California State University, East Bay (CSUEB) in 2006 saw the application of the HERC-UCB-developed data architecture models to modern human collections housed in UNAM Faculty of Medicine. Each of the institutions (UCB & UNAM) house more than the one skeletal collection, and the idea for building a meta-database (metabase) to query multiple skeletal collections quickly emerged. The FOROST initiative is the realization of this idea, and a partnership quickly grew from the founding institutions to include 15 museums and universities from 6 countries. The FOROST metabase now serves multiple high-resolution images from 260 specimens with distinctive bony traumas and pathologies.

FOROST already has more images of skeletal trauma and pathology than any printed volume, in significantly higher resolution, and all images can be accessed freely. This has far-reaching implications, because along with making access to forensic osteology images easier

for professionals and students in the developed world, FOROST provides access to many that would otherwise have no connection to reference imagery. Many images of specimens on FOROST are of research quality and many are accompanied by known medical histories or the known mechanism of trauma. FOROST-linked specimens and their images thus have the potential to be utilized as the basis for illustration, description, or other published work. This means that specimens served on FOROST can be cited, and the partner institutions and individuals who curate, describe, and photograph the material served in the metabase retain citable credit and copyright for the information and images provided.

The FOROST initiative goal is to develop a globally accessible metabase serving records and images of forensic osteology specimens that can be used as a reference for forensics workers worldwide. This introduction will provide guidance in both use and individual or institutional participation in FOROST.

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Metabase, Osteology, Image

H29 Analysis of Experimental Wood Chipper Trauma on Bone

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After attending this presentation, participants will understand the results of a study conducted to observe and analyze the skeletal trauma created by a home-model wood chipper.

This presentation will impact the forensic science community by providing information regarding trauma that may be suffered following dismemberment via wood chipper, thereby facilitating event reconstruction, victim identification, and conviction of perpetrators.

Since the highly publicized murder trial of Richard Crafts in 1989, there has been an explosion in pop culture media such as movies, television shows, and the internet postings involving body disposal using a wood chipper. It is repeatedly portrayed as a quick and effective method for disposing of a body and eliminating evidence while avoiding detection; however, in the scientific literature and research relating to human corpse dismemberment and mutilation, there are few studies focused on wood chipper trauma. This paper addresses these shortcomings through case reviews and a preliminary experimental study on wood chipper trauma to bone.

Five domestic pig (*Sus scrofa*) limbs were inserted into a light-capacity home model wood chipper, similar to models that are available for purchase at home improvement and hardware stores and most likely to be used in a criminal context. Following the insertion of each limb, the wood chipper receptacle was removed and the chipped materials were collected, weighed, photographed, and processed. After processing, chipped bone fragments of each limb were separated into five size categories using sieves with openings measuring 11.6mm, 5.85mm, 2.82mm, and 1.69mm respectively. Fragments from each category were weighed and examined for cut marks and other alterations.

Traumas observed had characteristics associated with sharp force trauma and blunt force trauma. Bone fragment sizes ranged from 45mm to less than 1mm. Only 13% of bone fragments by weight were collected in the largest sieve with 71% being collected in the second and third seized sieve. The chipping blade consistently cut the bone all the way through. A total of 239 bone fragments collected from the three larger sieves were observed to have at least one through-cut, and most had two such cuts, creating a roughly parallel-sided fragment. The average length between the two parallel cuts was 6.47mm. This characteristic is a product of the width and speed of the cutting blade as well as the speed and force with which the input chute or hopper brings the material in contact with the cutting blade. The consistent size and pattern of fragments produced are believed to be features that distinguish wood chipper trauma from other types of sharp force trauma.

The wood chipper used in this study produced square, V-shaped, and W-shaped alterations that were dissimilar to the cuts described above and most likely were not caused by the chipping blade. Possibly, these alterations were created when the bone came into contact with other metal components of the wood chipper, and may reflect components and defects specific to the wood chipper used. Other alterations observed included peeling, flaking, spurs, notches, and incomplete fractures. These and other non-cut alterations could potentially assist in associating fragments to a particular chipper model or perhaps to an individual chipper. Although not the primary focus of this study, several relevant soft tissue observations were also made. Of particular note was the fact that, while the bone material was consistently cut by the chipping blade, the skin remained largely intact.

This is a preliminary study, and additional tests such as using different models of chippers, different types of bones, different pre-chipping conditions (i.e., freezing or burning), and microscopic analysis of blade striations on the bone may be useful in further understanding wood chipper trauma patterns. Nonetheless, the study shows that wood chippers create a pattern of skeletal trauma that can be identified and associated to wood chippers in a forensic context. This pattern includes the production of bone fragments of a particular size, through-cuts resulting in roughly parallel-sided fragments, and other alterations that may be specific to the wood chipper used.

Skeletal Trauma, Sharp Force Trauma, Wood Chipper

H30 Patterns of Cranial Trauma in Korean War Remains at the Central Identification Laboratory (CIL)

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After attending this presentation, attendees will understand the variety of different peri-mortem cranial trauma observed in remains from the Korean War analyzed at the Joint POW/MIA Accounting Command-Central Identification Laboratory (JPAC-CIL).

This presentation will impact the forensic science community by describing peri-mortem cranial trauma that resulted from armed conflict and examining the correlation between different types of trauma and the circumstances of death.

The Central Identification Laboratory (CIL) and its predecessor organization, the Central Identification Laboratory-Hawaii, have identified a total of 209 Korean War casualties since 1982. In the

process, the CIL has generated a large corpus of Forensic Anthropology Reports on remains associated with the Korean War. In previous American Academy of Forensic Sciences (AAFS) presentations, the overall pattern of peri-mortem trauma in these remains was described and compared with wartime medical reports on trauma among United Nations casualties.[1,2] This presentation considers the cranial trauma observed in a sample of 56 individuals from both battlefield and Prisoner-Of-War (POW) recovery contexts. Peri-mortem fractures were recognized according to standard morphological and taphonomical criteria. When possible, ballistic trajectories were reconstructed from entrance and exit wounds. Blunt trauma was recognized by the lack of an entrance defect, as well as by plastic deformation. Unfortunately, the vagaries of taphonomy and recovery prevented an exact diagnosis of the form of trauma in every set of remains.

Eighteen of the 56 individuals exhibited non-specific peri-mortem fractures of the vault, face, or both; generally, the lack of a more specific interpretation is a result of incomplete preservation. One individual exhibited sharp-force trauma to the face. Six individuals, all from battlefield contexts, exhibited blunt trauma; one of these also exhibited ballistic trauma. Thirty-two individuals exhibited one or more ballistic defects or gunshot wounds. Of these 32, one was not well enough preserved to record directionality, while one was significantly fragmented by multiple projectiles and could not be reconstructed to determine their trajectories. Of the remaining 30, 14 exhibited a primary trajectory from an anterior origin, 12 from a posterior origin, and four laterally. Patterns of cranial trauma showed a clear correlation with postcranial trauma. The six individuals with cranial blunt trauma exhibited no postcranial trauma. Of the 14 individuals with anterior-origin ballistic trauma (one of whom also exhibited blunt trauma), only two exhibited postcranial fractures, and both were butterfly fractures of the distal right humerus that may have resulted from the individual's fall after being shot. Three of the four individuals with lateral ballistic entrances exhibited postcranial fractures to multiple elements which were consistent with either ballistic or blast trauma. Four of the 12 individuals with posterior ballistic entrances exhibited postcranial fractures consistent with either ballistic or blast trauma. Finally, of the 21 individuals that exhibited neither blunt nor directional ballistic trauma to the skull, 16 exhibited one or more postcranial fractures as well. It is possible that many of these individuals experienced blast trauma that fractured multiple elements with either no ballistic impacts or many small, irregular ones.

Previous studies have found the incidence of peri-mortem trauma in POWs to be significantly lower than in those killed in action. In this sample, only eight of the 56 individuals exhibiting cranial trauma were documented POWs. Two of these eight died soon after capture; one of the remaining five is known to have been killed by aerial strafing.

The views expressed herein are those of the author and not necessarily those of the Joint POW/MIA Accounting Command or the U.S. Department of Defense.

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Peri-Mortem Trauma, Cranial Fractures, Military Medicine

H31 A Return to the Basic Principles of Biomechanics to Interpret Blunt Force Trauma in Long Bones

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After attending this presentation, attendees will gain knowledge on basic biomechanical theories necessary to interpret blunt force injury in long bones.

This presentation will impact the forensic science community in addressing two common problems associated with fracture interpretation of blunt force injuries in long bones. The recognition and examination of the failure mode of bone (compression/tension/shear) is often lacking in trauma analysis and is reflected in common errors made in the field. One misconception assumes the location and direction of bone failure is directly related to the point of impact. Another is that analyses often concentrate on a single broken bone as opposed to evaluating the complete trauma pattern on the body.

Five types of external loading conditions are commonly described with long bone fractures:—compression, tension, shear, bending, and torsion. These external loading conditions are typically associated with recognizable fracture patterns, namely transverse, oblique, oblique transverse, butterfly, and spiral fractures. Fenton et al., as a reaction to recent anthropology literature, examined the association between these external loading conditions and resulting fracture patterns and demonstrated that blunt fracture patterns, particularly butterfly fractures, can be used to determine the point of impact on experimentally broken bones.[1] They further suggested four common fracture patterns are recognized: incomplete butterfly fractures (tension wedges); transverse fractures (initiation); failure angle shifts of 45 degrees; and breakaway spurs. Fracture pattern "categories" linked to external loading conditions may lead an expert to a factual diagnosis regarding the point of impact, but the failure mode of the material (tension/compression/shear) is ignored and, as such, the forensic scientist may misinterpret the external loading conditions and/or the point-of-impact.

"Point-of-impact" is commonly used to describe and to interpret the wing of a butterfly fracture. Yet without soft tissue or a corresponding tool mark on bone, biomechanically, a butterfly fracture only indicates the direction of bending failure.[2] The area of failure in a bone shaft and the location and direction of impact are not one and the same concept. In any tubular bone, the failure mode only provides information as to the direction in which the bone bends (directionality) and to an anatomical weak point of the shaft. Furthermore, the failure modes involved in bending bone (tension, shear, and compression) are not mutually exclusive; thus, any failure mode can be used to establish bending direction and failure in long bones.[2] Therefore, a long bone fracture is attributed to the biomechanics of fracture production and not to a reconstructed fracture pattern or to a known impact site.[3] Despite the findings of three-point impacts in the laboratory, real-world scenarios such as automotive collisions, falls from heights, or beatings may result in long bone fractures via a variety of external loading conditions. Thus, a total body trauma pattern must be considered when examining and interpreting any fracture.

Complicated fracture patterns may be unraveled when a proper stress analysis of bone is conducted to determine stress/strain distributions as well as external loading and boundary conditions; all fractured surfaces are macro- and microscopically examined; and a complete analysis of body trauma is performed. For an accurate diagnosis, these steps need to supersede specific fracture interpretations. In this presentation, three cases of blunt traumatic injury to bone will be illustrated. While the reconstructed bone failure patterns are clear in each case, closer examination of biomechanics, along with

knowledge of the overall trauma pattern, reveals clues as to the mechanism of failure. With the use of these cases, the above-mentioned pitfalls of trauma analysis within tubular bone are addressed and clarified.

Forensic anthropologists and pathologists are encouraged to closely examine bone failure at the materials level; to observe stress risers and resistors involved in a bone injury; to consult biomechanical engineers to evaluate loading conditions and internal stress distribution in long bones; to correlate fracture morphology and failure mode evidence in the fracture pattern; and to avoid over-reaching assumptions and predictions regarding impact sites. The proposed approach is designed to reduce error in our contributions to cause and manner of death and to avoid the negative repercussions associated with an inaccurate trauma analysis.

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Bone Trauma, Failure Mode, Point-of-Impact

H32 Skeletal Dismemberment Analysis Utilizing Surface Metrology

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The goal of this presentation is to explain the potential applications of 3D computer modeling and surface metrology to saw-related dismemberment of skeletal remains. Specifically, the correlation of the surface variables Pv and Pp to saw tooth deviation will be demonstrated.

This presentation will impact the forensic science community by showing how it will increase the range of saw characteristics that can be gleaned from their resulting kerfs, and allow for more accurate and confident identifications of the tools used in a dismemberment.

Saw kerf examination is a vital part of any investigation of dismemberment as it provides many clues to the characteristics of the saw that was used. The accuracy and confidence of the methods used in this examination are vital, as they must now hold up to *Daubert* standards of scientific evidence in court. Symes was the first to create real standards for saw kerf analysis, and established many of the still-used characteristics of saw kerfs in bone that can be tied to their acting implement.[1]

Over the last two decades, other researchers including Bailey, Freas, and Saville have attempted to reduce the characteristics of saws that create changes in kerfs on bone to their most fundamental elements. These studies have confirmed the ability to tie kerf features to saw types within levels of statistical confidence. This research, however, has focused largely on increasing magnification of kerf walls, or ignoring the kerf wall all together, and examining so-called false starts or incomplete kerfs.[2-4] Very little research has been done on the potential discriminatory uses of peaks and valleys of the kerf wall itself.

The present study examined experimentally created kerf walls on twenty-five pig femora. Each kerf was created by one of a group of hacksaw blades meant to represent the variability seen in this type of sawing implement. Stereo images were taken of each of the kerf walls using a Leica MZ16 and combined to create a 3D computer model. These models were then examined using the programs StereoExplorer and SFRAX 1.0 where thirty different variables were collected that describe changes in the kerf wall surface.[5-6] These measures were then compared to quantifiable features on the saws for any correlations.

Of the measured saw characteristics, saw set was prioritized because of its presumed affect on the kerf wall striations. A strong correlation (R^2>0.70) was found between the amount of saw set deviation and the depth of the kerf wall striations. Previously, the saw set was inferred solely from the kerf width. The presented method provides a new avenue to evaluate this feature of the saw. This measurement is sensitive to variation between the right and left sides of the saw; therefore, it can be used to identify individual characteristics of the saw resulting from manufacturing deviations. This study shows the potential application of 3D computer modeling for saw kerf analysis as a method not only for gathering more quantifiable information from kerfs, but also for changing the traditional ways in which kerfs are viewed and analyzed.

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Dismemberment, Surface Metrology, Kerf

H33 The Difficult Task of Assessing Peri-Mortem and Postmortem Fractures on the Skeleton: A Blind Test on 210 Fractures of Known Origin

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The goal of this presentation is to highlight the difficulties and pitfalls a forensic anthropologist has to face when diagnosing bone fractures as peri-mortem and postmortem.

This presentation will impact the forensic science community by showing how morphological and macroscopic evaluation on bone fractures has to be handled with care since the evaluation of a bone fracture as being peri or postmortem may be difficult, treacherous, and, at times, observer-dependent and thus influenced by knowledge, intuition, and training of the observer. If postmortem fractures can be detected more easily, on the other hand, peri-mortem fractures can be wrongly identified, especially when spongy bone is involved and when time has taken its toll on the remains.

In the field of forensic anthropology, differentiating between peri-mortem and postmortem fractures is one of the most difficult challenges. Indicators of peri-mortem injury have been identified (i.e., "green" characteristics of the fracture or fracture margin color), but most forensic anthropologists realize that determining between peri-mortem and postmortem fractures may be impossible. Few studies have focused on the error rate associated with classifying a fracture as peri- or postmortem. How many times will a postmortem or taphonomic fracture be mistaken for a peri-mortem fracture and vice versa? Which bones present the most difficulty?

The study goal was to evaluate the error rate associated with differentiation between peri-mortem and postmortem fractures. The study method was a blind test of two anthropologists with seven and three years of experience, respectively. Each anthropologist independently examined 210 fractures of known origin and classified the fracture as peri-mortem or postmortem.

Four skeletons were selected from a skeletal collection of 250 individuals who died in 1991 and whose skeletons were exhumed in 2001. These decedents were unclaimed and thus available for scientific research according to Italian Mortuary Police Regulations. Of these four skeletons, one had died of natural causes and three were pedestrians struck by motor vehicles: tram, car, and truck. All three motor vehicle accident victims had numerous blunt force, soft tissue, and skeletal injuries, that were documented during the autopsy.

For all cases, the number and sites of bone fractures documented during the 1991 autopsy were recorded as well as fractures observed on the exhumed remains. Fractures identified during the autopsy were designated as peri-mortem fractures. Fractures observed on the exhumed remains, but not documented during the autopsy were classified as postmortem fractures. The total number of fractures was 210. The two observers blindly scored all 210 fractures on the four skeletons as peri-mortem, postmortem, or uncertain. The results were analyzed by comparing the expected classifications with the observed classifications.

The results showed the observers were more accurate when classifying a postmortem fracture (75% accurate) compared to peri-mortem fractures (~45% accurate). Also, scoring postmortem fractures was easier than peri-mortem fractures; 16.5% of peri-mortem fractures and 7% of postmortem fractures were identified as uncertain. Bones with little cortical bone such as ribs and innominates were more difficult to score than long bones and skull bones.

Globally, this study illustrates the difficulty of differentiating peri-mortem from postmortem fractures in buried human remains. The correct identification of peri- and postmortem fractures is crucial to reconstructing the circumstances surrounding death, but macroscopic and morphological criteria are limited, and sometimes misleading. The study results serve as a cautionary note concerning interpretation of peri- and postmortem fractures as well as an invitation to search for novel methods of analysis (i.e., histology, immunochemistry, electronic microscopy) for differentiating peri- and postmortem fractures.

Blunt Force Trauma, Peri-Mortem, Postmortem

H34 Retrospective Study of Skull Fractures Observed Following Terminal Falls

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The goals of this presentation are to review the current literature of skull fracture biomechanics and to evaluate the variation in skull fracture patterns observed in the medical examiner population.

This presentation will impact the forensic science community by increasing the general knowledge of skull fracture types associated with terminal falls.

Skull fractures are common in the medical examiner population. At times, the circumstances surrounding the death are unknown and skull fracture pattern interpretation plays a significant role in the manner of death classification. When faced with a complex skull fracture pattern, pathologists often look to forensic anthropologists for guidance in fracture pattern interpretation.

Recent research has focused on blunt force trauma to the head and associated fractures. For example, Hamel et al. developed a multibody study using finite elemental modeling to identify the parameters that influence the mechanism of skull fractures.[1] They concluded that two fall parameters, impact velocity and impact surface, and two biological parameters, cortical thickness and cortical rigidity, had the greatest influence on the mechanism of skull fractures. Additionally, Kremer and Sauvageau evaluated the relationship between three criteria Hat Brim Line (HBL) rule, side lateralization, and number of scalp lacerations) and the mechanism of injury.[2] Also, they evaluated the predictability of the mechanism of injury by combining the criteria. The authors conducted a six-year retrospective study of autopsy cases with skull fractures. They found that fractures resulting from falls tended to be within or below the HBL, on the right side, and have three or less scalp lacerations. Fractures resulting from homicidal blows tended to be above the HBL, on the left side, and have more than three scalp lacerations. When combining two criteria in favor of a fall, the predictive value of the mechanism was 65.9%. When combining two criteria in favor of a homicidal blow, the predictive value was 100%. The predictive value increased to 83.3% when three criteria in favor of a fall were combined.

Although the research has furthered the understanding of skull fracture dynamics, one feature of a skull fracture is being overlooked: fracture type. In children, fracture type has been used to differentiate between accidental and inflicted trauma.[3] Similar research investigating the correlation between fracture type and cause of injury needs to be performed in adults. As a result, the Harris County Institute of Forensic Sciences conducted a retrospective pilot study of skull fracture types observed in accidental deaths. The study included five years of records.

During the study period, 192 deaths were classified as accidental with fracture listed in the cause of death. Of these, 57 cases were autopsied and 46 cases had skull fractures documented during the autopsy. Thirty cases had skull fractures that resulted from terminal falls at or near a standing height. Reviewing autopsy photographs of these cases, the fracture types were coded as linear in 23 cases and as stellate or comminuted in seven cases. In comparison, 12 cases involved a fall from a significant height, such as a fall from a roof; again using autopsy photographs, ten of these fractures were coded as comminuted and two as linear. The occurrence of skull fracture types for the two groups, standing height falls and significant height falls, was shown to be significantly different using a Mann-Whitney test (p=.000). Although the sample size was small, the results indicate that fracture type may be an important variable in differentiating terminal falls from a standing height and other mechanisms of injury.

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Skull Fractures, Fall, Fracture Types

H35 Craniometric Sex Determination in the Modern Thai Population

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The goal of this presentation is to introduce population-specific discriminant functions for sex determination in the modern Thai population and provide an understanding of patterns of sexual dimorphism within this population.

This presentation will impact the forensic science community by filling a gap in currently existing normative craniometric databases, and providing novel, statistically robust, population-specific discriminant functions for sex determination when analyzing unidentified individuals known or believed to be of Southeast Asian, specifically Thai, ancestry.

Metric approaches to the analysis of human variation are among the oldest scientific traditions in the field of physical anthropology.

Despite an ignoble intellectual adolescence, which saw craniometrics applied tacitly and overtly in support of racist sociopolitical and pseudoscientific agendas, these methods have matured into a robust and respected part of modern physical anthropology's analytical engine. Craniometric studies now find application throughout the whole of the discipline. Forensic anthropology is no exception, as craniometric analyses are essential tools for the determination of sex and ancestry in unidentified human skeletal remains. Yet the validity of such analyses depends upon having population-specific reference datasets of sufficient robusticity to adequately characterize the true range of morphological variation within a given population. This is especially true of craniometric discriminant function analyses (the preferred metric method for sex and ancestry determination), in which both the classification of an unknown individual and that classification's statistical certainty (in terms of posterior and typicality probabilities) is driven entirely by patterns of variation within and among the reference populations used to generate the discriminant functions. Reliable sex determination standards that are appropriate for the population(s) under consideration are of particular importance to forensic anthropology, as the determination of all other parameters of the biological profile is predicated on an accurate assessment of an unknown individual's sex. Similarly, reliable standards for ancestry determination are critical to identification efforts, as ancestry is arguably the second-most dominant criterion, after biological sex, by which individuals are classified and identified.

The 2004 Indian Ocean tsunami, which killed nearly 250,000 people in South and Southeast Asia, including approximately 8,200 in Thailand, is but one of a series of natural disasters and human rights violations that have laid bare the need for regionally-based, population-specific forensic anthropology standards and normative datasets. With regards to Southeast Asia, several studies have demonstrated significant patterns of craniometric variation among local populations within the region; however, to date, only a few scientific articles addressing the need for Thai-specific forensic standards have been published in the international forensic literature. Importantly, these studies clearly demonstrate that the available American standards are inaccurate when applied to Thai populations and, in such applications, lead to systematic misclassifications of Thai males as females, due to the relative gracility of the Thai population as compared to American populations. Nevertheless, these studies are an insufficient remedy to the lack of appropriate population-specific standards for the Thai, as they primarily address sex determination from postcranial skeletal elements, which are commonly regarded as less informative and reliable than the more robust craniometric analyses (but cf. Spradley and Jantz 2011).[1]

To address these issues, 20 standard cranial measurements were collected on a large sample (n=385; 262 males/123 females) of modern Thai skeletons from three medical school anatomical collections (Chiang Mai, Khon Kaen, and Naresuan Universities). These craniometric data were used to generate two separate linear discriminant function equations for sex determination: (1) using the full set of 20 craniometric variables; and, (2) using eight forward stepwise-selected variables to generate a function potentially applicable to incomplete crania. In both analyses, the discriminant functions were found to be most strongly driven by the larger, more generalized dimensions of the cranial vault and facial skeleton that contribute significantly to the overall size differences between males and females. This mirrors the patterns of variable selection observed in other craniometric studies, and indicates the broad pattern of craniometric sexual dimorphism within the Thai population does not differ significantly from other ancestry groups. Additionally, these functions have cross-validated correct classification rates of 82.6% and 82.9%, respectively. These results are similar to the performance of other population-specific sex determination standards, indicating the validity and broad utility of these functions to forensic anthropology, both in Thailand and the United States.

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Craniometrics, Sex Determination, Southeast Asia

H36 Forensic Significance Beyond Taphonomic Characteristics: Using Archaeological Context, Craniometrics, and Radiocarbon Dating

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The goal of this presentation is to demonstrate archaeological, craniometric, and radiocarbon approaches to assessing forensic significance to supplement the standard taphonomic interpretation.

The presentation will impact the forensic science community by addressing the often difficult task of assessing the forensic significance of skeletal remains and by providing alternative approaches beyond simply interpreting taphonomic characteristics.

In the absence of primary contextual information, the assessment of forensic significance of remains found in a medicolegal context becomes difficult and often focuses on the taphonomic characteristics observed on the remains. This presentation follows a case study of remains removed from their primary context prior to medicolegal involvement and shows alternative approaches to assessing forensic significance that may be used to supplement the standard taphonomic interpretation.

In the summer of 2011, skeletal remains were discovered in the Greenpoint section of Brooklyn, New York. The remains consisted of a partial cranium, partial mandible, and two cervical vertebrae, which were discovered during outdoor construction in a residential backyard. The remains were removed by the property owner prior to the investigation; however, the general area remained undisturbed. The property owner was not able to provide additional details at the time of the investigation and the temporal provenience of the remains could not be assessed. In order to assess the forensic significance of the remains, the archaeological context of the immediate vicinity was documented and interpreted, craniometric data were assessed with respect to secular variation, and isotopic values were examined with respect to bomb-derived radiocarbon.

Archaeological excavation of the scene provided data used to reconstruct the context and determined that the remains, and associated artifacts, likely came from a secondary deposit within a shallow man-made depression. The associated diagnostic artifacts included: glass bottle fragments, a ceramic pipe stem fragment, a ceramic button, and a machine-cut copper nail. Although the pipe stem may date to the 18th or 19th-century, most of the artifacts were in use during the early 20th-century. The absence of modern debris within the discreet deposit suggests that the deposit was likely created more than 50 years ago.

Previous research has noted a secular trend toward higher, longer vaults and narrower vaults and faces in American crania and that cranial variables exhibit a high correlation with the birth year of the individual.[1,2] Accordingly, an analysis of the craniometric variables may assist in addressing the temporal origin of unknown skeletal remains. In this case, discriminant function analyses were performed using FORDISC 3.1 on the available measurements of the cranial vault using "19th-century" groups from the Terry and Todd Collections and "modern" groups from the Forensic Databank.[3] When tested against modern groups, the skull did not classify as being a typical member of any of the groups. When tested against White and Black groups of both sexes from the 19th-century and modern groups, the skull grouped most closely with Black females from the 19th-century.

Samples from the right parietal and left lower canine were extracted for radiocarbon analysis. Results indicated that the remains did not contain bomb-derived radiocarbon clearly indicating the individual lived and died before 1955. More precise radiocarbon dating in the five centuries before 1950 is hindered by natural fluctuations in environmental radiocarbon levels, but this case presented the possibility for refinement. The radiocarbon level in tooth dentin was found to be statistically different from that in enamel. Tooth dentin remodels throughout life but tooth enamel does not, so differences in radiocarbon

levels can appear as a person ages. Here, the osteological age estimate of the individual was coupled with the enamel and dentin measurements in order to derive an environmental radiocarbon level rate-of-change. The derived rate-of-change was then matched to the known environmental rates-of-change over the past five centuries and, thus, the remains were tentatively assigned to a more specific time frame than would otherwise be possible. In this case, two solutions were found; either the 17th-century or early 19th-century.

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Significance, Taphonomy, Radiocarbon Dating

H37 Assessment of Presentation Methods for ReFace Computerized Facial Approximations

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After attending this presentation, attendees will understand that the way in which a facial approximation is presented to the public impacts the probability of achieving a successful identification of the unknown individual with certain presentation methods having a greater likelihood of eliciting identification success. Attendees will also be made aware of the possibility that traditional methods of evaluating facial approximation success may not be the most appropriate when considering the practical applications of these approximations.

This presentation will impact the forensic science community by recommending new methods of presenting facial approximations to the public in order to improve recognition potential. It will also provide new criteria for evaluating the performance of facial approximations in a way that is more representative of their practical application.

New computerized techniques have made it possible to present facial approximations in a variety of layouts, but there are currently no clear indicators as to what style of presentation is most effective at eliciting recognition. The primary purpose of this study is to determine which of the tested presentation methods produces the most favorable recognition results. A secondary goal of the research is to evaluate a new method for testing the accuracy of facial approximations. Previous studies have evaluated facial approximation effectiveness using standards similar to studies of eyewitness positive identification in which a single, definitive choice must be made by the research participant. These criteria seem inappropriate given the accepted understanding that facial approximations cannot produce positive identifications but are merely an investigative tool leading to positive identification.

Facial approximations were generated using CT scan data from living volunteers instead of scans from skulls of actual missing persons. Approximations were presented in one of five formats: Basic, Front and Profile, Weight Variation, Estimated Average Age, and Estimated Age Range. Participants were asked to compare one approximation at a time to one front and one profile photograph of the "missing person." If the participant felt the approximation looked enough like the missing person that they would contact authorities, the approximation would be placed in a folder marked "Yes." If they did not feel there was enough similarity to warrant contacting police, the approximation would be placed in a "No" folder. Once all ten approximations had been evaluated, the participant was asked to simultaneously compare all approximations in the "Yes" folder with each other and the missing person photos to determine which approximation looked most like the missing person.

Effectiveness of presentation methods was determined using three values: sensitivity (percent of true positive responses out of the total possible true positives), specificity (percent of true negative responses out of the total possible true negatives), and percent above chance for correct final selection (the criterion used by most other facial approximation studies). Functionally, a successful facial approximation is one that generates a short list of possible candidates, including the missing person in question. For this reason, and the fact that false positives can be ruled out with further investigation, sensitivity was chosen as the primary benchmark for success.

Weight Variation achieved the highest sensitivity, but this value was not significantly different from Front and Profile or Estimated Age Range. Interestingly, the two methods with the poorest performance each consisted of only a single picture, leading to the possibility that presenting more than one image of an approximation may improve recognition potential. When all criteria were considered, Front and Profile produced the best results. This is particularly encouraging because this presentation method can easily be achieved with traditional clay approximations as well. Also, it was found that percentage above chance for final selection failed to reflect the successfulness of particular presentation methods, and generally under-represented the successfulness of all methods, in terms of sensitivity. These results suggest that single-selection studies may not be the best method for determining the effectiveness of facial approximations.

Facial Approximation, ReFace, Method Performance

H38 Craniofacial Regional Variation in the United States: A Geometric Morphometric Study

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After attending this presentation, attendees will understand how environmental adaptation has impacted cranial variation in the United States within the last hundred years.

This presentation will impact the forensic science community by providing insight into craniometric variation as it applies to issues of cranial plasticity, migration, and genetic affinity. In addition, through the use of modern quantitative methods, this study will present a broader understanding of genetic versus environmental influences on cranial variation.

The main purpose of this study is to explore the effect of different environments within the United States on cranial variation. Two modern human samples were used in this analysis: 36 individuals from the donated New Mexico Unidentified and 31 individuals from the C.A. Pound Human Identification Laboratory (CAPHIL) at the University of Florida. The CAPHIL individuals represent populations residing along the eastern coast of the United States, while the New Mexico Unidentified represents populations in the west coast. Only European-American individuals were used in this analysis to establish a genetic baseline for cranial variation. Three-dimensional coordinate data for 21 landmarks were collected for individuals from these collections and a software program was used to run all multivariate statistical analyses. In order to reach a consensus least-square fit and acquire new shape coordinates for the entire dataset, Procrustes' superimposition was used to translate, scale, and rotate the landmark data. Principal Components Analysis (PCA) was performed to explore patterns of variation among the data in muliti-dimensional space, allowing the variables to be reduced to a few dimensions that represent the majority of cranial variation. The PCA identified seven principal components that represent 81% of the total variation. Typically, in closely related samples, the number of principal components representing total variation would be reduced; however, the larger number of principal components for this analysis is expected if environment can affect cranial shape.

To identify the landmarks responsible for the variation, Canonical Variate Analysis (CVA) was employed. The CVA produced two significant canonical variates with the first representing 91% of the

variation, which included landmarks in the posterior and base portions of the crania (e.g., opisthion and asterion). In addition, Multivariate Analysis of Variance (MANOVA) was used to measure the statistical significance of the variance between groups. The MANOVA found significant variation in both centroid size (p-value=*0.049)* and shape (p-value=*0.001*) for the three groups. This suggests significant regional variation. Discriminant function analysis was also performed to discern the approximate level of separation by measuring the degree to which individuals could be correctly allocated to their location. A correct classification rate of 74% was found from the cross-validation and suggests these individuals are easily classified due to variation in cranial shape. Lastly, Mahalanobis D^2 was calculated to measure the distance between populations. The Mahalanobis D^2 was found to be 1.969 between the east and west coast groups. The two east coast groups were found to have a distance of 0.826. The distances found suggest a larger degree of East-West separation in European-Americans.

The differences seen in this study may affect ancestry assessment in different parts of the country and warrant further study. However, most of the variation was observed in the cranial vault and base suggesting facial landmarks are less easily influenced by the environment.

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Geometric Morphometric, Ancestry, Regional Variation

H39 Sexual Dimorphism in Complete and Fragmentary Navicular Bones

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After attending this presentation, attendees will understand how to use the navicular bone to aid in the estimation of sex in unknown individuals.

This presentation will impact the forensic science community by showing new measurements that will aid in the identification of sex in fragmented remains.

Accurate sex estimation is an essential step in the identification of unknown skeletal remains. Expanding sexing methods to include previously unused or underused bones adds to the battery of available techniques and can increase the accuracy of such assessments. Such novel techniques may also be invaluable when sexing incomplete remains. In 1976, Steele was one of the first researchers to examine sexual dimorphism of the talus and calcaneus, and since then other researchers have repeated the work and confirmed that the talus and calcaneus are useful in determining sex with accuracies as high as 96%.[1-10]

These researchers have not only shown that the accuracy rates of the talus and calcaneus are repeatable, but also that the techniques can be applied to different populations from the past and the present. Other than the talus and calcaneus, little research has been done on the remaining five tarsals. In 2009, Sheena Harris, using The William M. Bass Skeletal Collection and a mini-osteometric board, measured the maximum length and width of all seven tarsal bones.[11] However, her measurements required the bones to be complete and she did not look at the smaller dimensions on the bones. In 2011, it was demonstrated that the three cuneiforms, whether complete or fragmented, are useful for sex determination.[12] This finding has shown there is a need for investigating the other tarsal bones for this purpose.

The current study examines the navicular bone from 100 adult individuals (50 male and 50 female), both Blacks and Whites, from the William M. Bass Skeletal Collection for their possible use in sex determination. This study first examined the "maximum" measurements following Harris from complete navicular bones and then created additional original measurements (such as measurements involving specific articular surfaces and tubercles) that divide the bone into smaller segments.[11] Digital sliding calipers were used to take 11 measurements (three following Harris and eight new measurements) of the left navicular bone.[11]

FORDISC 3.0 was used to perform Discriminant Function Analysis (DFA) in order to test multiple measurements for their efficiency in sex determination.[13] The results of this study (78.8% accuracy for the maximum height, 78.6% for maximum width, and 86.6% for maximum length) show that while Harris' maximum measurements are fairly accurate, they may not be accurate enough to be used as the only deciding factor in sex determination, with the exception of the maximum length. Further, when a bone is incomplete, substitute measurements are also useful for sex determination. This study also demonstrates that if more than one measurement is present, all measurements should be included. Following the baseline suggested by Scheuer and Elkington, measurements (univariate) with an accuracy rate of 80.0% or greater were considered to be useful.[14] Four of the 11 variables used in this study meet or exceed that threshold. When multivariate approaches are taken into account, accuracies may be even higher. For instance, when all 11 measurements are used in a DFA, the overall accuracy rate is 87.4%. If the relative weight of these measurements are taken into account and the measurements with the lowest contributions to the model are systematically dropped, the accuracy then rises to 90.3%. While some of the individual variables have accuracy rates below 80%, the rate increased above 80.0% when combined with at least one other measurement. In conclusion, although the navicular is a small bone of the foot, it can be useful in sex determination whether the bone is complete or incomplete.

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Navicular, Sexual Dimorphism, Bones

H40 Estimating Adult Stature From Metatarsal Length in a Galician Population (NW of Spain)

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After attending this presentation, attendees will understand the potential contribution of new formulas to estimate adult stature from metatarsal length in a Galician population.

This presentation will impact the forensic science community by presenting new formulas and demonstrating how they can increase the accuracy of adult stature estimation, especially when long bones are not present or are fragmented.

While it is accepted that long bones provide a more accurate estimation of stature, in practice fragmentary remains make the task difficult. However, small bones, such as those of the hands or feet, are very often well preserved in a variety of contexts and, for this reason, it is necessary to develop methods for estimating stature from these elements. Although the formulas developed by Byers et al. have been used in the forensic arena for more than 20 years, it is clear that it is the reference population from which they were derived which limits their utility for use with other populations.[1] To verify the use of the dimensions of the metatarsals as estimators of adult height in our population, a method for estimating the stature of Spanish adults using radiologically determined metatarsal lengths has been developed.

Method: The present research is based on a study of 101 (36 males and 65 females) healthy Caucasoid volunteers, over the age of 25 years from Galicia (NW of Spain). All persons with skeletal deformities, pathologies, or fractures which could preclude accurate measurements were excluded from the study. Height was determined with a measuring rod, placing the volunteer barefoot, erect, and looking up, with the back against a graduated ruler. The first and second metatarsals of the left foot of the 101 volunteers were measured by a dorso-plantar X-ray using a digital medical image viewer with a Radiological Archive and Image Management (RAIM) application 1607 JAVA from UDIAT, commonly used in hospitals. Although digital measurement is calibrated by the system itself, in order to minimize errors, a metallic ruler was used to confirm precision. All measurements were obtained twice and registered in millimeters. Statistical analysis of the data was carried out using the R environment (www.r-project.org).

Description of the measurements:

- M1—Maximum length of 1st metatarsal—the distance between the tip of the tuberosity and the most distal point of the head.
- M2—Maximum length of 2nd metatarsal—the distance between the proximal tip and the most distal point of the head.

Results: The highest correlation obtained (R=0.755) was with the maximum length of the 1st metatarsal for males. The corresponding regression equation is as follows: S=756.336 + 13.686*M1. When the equation is applied to both the first and second measurements, no significant differences in the correlation coefficients were detected. Additional formulas and summary performance statistics will be presented. It is important to note that participants in the study were adults and, thus, these formulas should not be used for estimation of stature for individuals younger than 19 years old.

The addition of metatarsal data for the estimation of stature has proved to be a valuable contribution in assessing the biological profile of Spanish adults. Additionally, because the equations are based on radiographic measurements of the fleshed foot, they may be of use for mass disaster, dismemberment, or other non-skeletonized cases where the soft tissue of the foot is still present.

Reference:

1. Byers S, Akoshima K, Curran B. Determination of adult stature from metatarsal length. Am J Phys Anthropol 1989; 79(3):275-9.

Estimating Adult Stature, Metatarsal Lengths, Dorso-Plantar X-Ray

H41 An Examination of Postmortem Interval Relative to Microbial Biomass of Soil at the MSU Forensic Science Research Facility Plot

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After attending this presentation, attendees will be informed of the Mississippi State University (MSU) Forensic Science Research Plot and the interdisciplinary research taking place at the facility. The focus of this presentation will be on recent controlled decomposition studies examining the relationship of Postmortem Interval (PMI) to the microbial biomass of soil and bone.

This presentation will impact the forensic science community by contributing new data to the expanding knowledge base of microbial biomass in relation to PMI of various sample types (e.g., bone, tissue, and soil) and provide a new dataset to compare to recent studies at the Anthropological Research Facility in Tennessee as well as other research units across the United States.

The MSU Forensic Science Research Plot conducts interdisciplinary studies aimed at understanding PMI, decomposition, and taphonomic processes in Mississippi. Collaboration with the Mississippi Agricultural and Forestry Experiment Station (MAFES) allows access to non-euthanized porcine (*Sus scrofia*) samples and enables controlled research throughout the year.

To develop a baseline biomass reference sample, three porcine experiments were monitored throughout active decomposition to mummification/skeletonization. The pigs were enclosed in fencing to reduce scavenging which may alter soil composition. Basic observations and notes were maintained, as well as serial photography, and included into the Porcine Taphonomic Database. Two juvenile samples (approximately 25 – 30 pounds) were placed at the facility during the fall of 2011 and became mummified/skeletonized within two weeks due to high temperatures. The third sample (approximately 200 pounds) was placed in the winter of 2012 and became partially mummified and skeletonized throughout the spring. The high fat content, as well fluctuating temperatures, extended the decomposition interval. Prior to placing the pigs, control soil samples were collected. Daily soil samples were collected beneath the center of each specimen during active decomposition. As a final step, rib samples were collected and pulverized (200mg) for quantitative PCR analysis.

A quantitative real-time PCR for the evaluation of bacteria and fungi from each sample was performed. Standard quantification curves were created with universal primers targeting a 200-base pair fragment of the 16s rRNA gene from *E. coli* and a 300-bp fragment of the fungal ITS region from *Fusarium solani*. Five serial dilutions of known concentrations of PCR products were generated and run in triplicate for a single standard curve and used to estimate DNA concentration from the unknown samples.

With the same primers, test soils, bone specimens, and standard curve samples were analyzed. Asymmetrical cyanine dye was used for bacteria and a fluorescent DNA binding dye was used for fungi. Quantitative-PCR efficiencies of 94% and 77% were obtained for bacterial and fungal amplification, respectively.

Due to the climate in Mississippi, decomposition is generally accelerated and, therefore, bacterial growth is accelerated. Bacteria

naturally reproduce rapidly (every 15 to 20 min) in soil and can be greatly influenced by nutrient availability and physiochemical environment, such as temperature and moisture. The first two pigs displayed a dramatic increase in bacteria within the first week of decomposition and then declined. Pig 1 became mummified and experienced a gradual decrease in bacteria while Pig 2 became skeletonized with a rapid decline of bacteria. The differential results may be due to lingering nutrients provided by the mummified remains. Pig 3 is currently being analyzed and the results are expected to mirror the results from Pig 1 and 2.

Although quantitative PCR has been established as a useful tool for measuring microbial biomass as a means for determining PMI, more regional studies are needed to better understand the relationship between bacteria and decomposition in different ecological settings. Researchers with the MSU Forensic Science Research Plot have collaborated with the National Museum of Health and Medicine to develop a baseline reference sample for Mississippi. Ultimately, understanding the rates of change in bacteria throughout the decomposition process will aid in determining PMI in future forensic cases.

Forensic Taphonomy, Postmortem Interval, Microbial Biomass

H42 Assessing the Efficacy of Basicranial Angle to Determine Ancestry

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After attending this presentation, attendees will understand the potential application of using cranial base angle to determine ancestry of unknown human skeletal remains.

This presentation will impact the forensic science community by providing a new method to aid in the identification of fragmentary skeletal remains.

A primary task of the forensic anthropologist is to construct a biological profile of unknown human skeletal remains. The skull is recognized as providing the highest accuracy for determining ancestry. However, portions of the skull that are most diagnostic, such as the face, may be damaged or missing, particularly when a skeleton is discovered in an outdoor context. In such situations, data used to construct the biological profile may be limited. The cranial base is centrally located in the head and protected by soft tissue which may prevent bony destruction. While the basicranium is a popular region for evolutionary studies, it has not been used as frequently as an identifying feature in forensic contexts. Holland achieved 70% – 90% correct classification of White and Black males and females utilizing multiple linear regression models.[1,2]

Wescott and Moore-Jansen later found the measurements used in that study have high interobserver error rates.[3] McKeown and Wescott attained 85% correct classification using geometric morphometric methods for determining ancestry utilizing cranial base landmarks.[4] The current study examines basicranial flexion in modern human populations to determine if it is a useful indicator of ancestral group affiliation.

A total of 196 males and females of European American and African American ancestry from the Hamann-Todd Human Osteological Collection and the Robert J. Terry Anatomical Skeletal Collection were utilized in this study to test the null hypothesis that there is no difference in mean basicranial angle between ancestral groups. Nasion-sella length, basion-sella height, and basion-nasion length were measured and then used to calculate the cranial base angle at sella using the Law of Cosines. Interobserver and intraobserver error tests were conducted to determine if the measurements are repeatable. Intraobserver error is equal to or less than 1.20mm for each measurement and interobserver error averaged less than 1.83mm for each measurement.

There is a statistically significant difference in mean basicranial angle between European Americans and African Americans (t=2.49, p-$value$ <0.05). Analysis of covariance indicates that ancestry is the sole factor influencing basicranial angle while collection, sex, and age at death have no significant effect. Logistic regression analysis was employed to calculate the odds that an individual belongs to an ancestral group, producing the model *log(odds)=6.1233 + -0.0451*cranial base angle*. The probability that the angle is African American is given by the formula *P=1/(1 + e^log(odds))*. Individuals with a cranial base angle greater than 140.2° are more likely African American, whereas individuals with a cranial base angle less than 131.3° are more likely European American. Individuals with a cranial base angle between 131.4° and 140.1° cannot be classified with certainty greater than 0.55. A receiver operating characteristic curve analysis was performed to assess the sensitivity and specificity of the test at multiple levels. The probability that the ancestry classification for a randomly chosen positive case (African American) will exceed the result for a randomly chosen negative case (European American) is 0.617. Eighty-eight individuals could not be classified using the established cut-off rule. Of the remaining 108 individuals, 66% were correctly assigned to their ancestral category.

This study demonstrates that cranial base angle can be used to estimate ancestry of unknown skeletal remains. The current method only requires one to identify three cranial landmarks and record three measurements to calculate cranial base angle using sliding and spreading calipers. The calculated angle can be used to provide a probability that the specimen belongs to a particular ancestral group. The only difficulty in applying this method is accessing sella to measure anterior and posterior cranial base lengths. The vault must be absent if using sliding calipers or else a medical imaging modality must be used. Overall, this method is particularly useful for fragmentary remains to aid in the construction of the biological profile and should be used in conjunction with other metric and non-metric methods. It must be tested on an independent sample to further judge its classificatory power.

References:

1. Holland TD. Race determination of fragmentary crania by analysis of the cranial base. J Forensic Sci 1986a:31(2):719-25.
2. Holland TD. Sex determination of fragmentary crania by analysis of the cranial base. Am J Phys Anthropol 1986b:70(2):203-8.
3. Wescott DJ, Moore-Jansen PH. Metric variation in the occipital bone. J Forensic Sci 2001:46(5):1159-63.
4. McKeown AH, Wescott DJ. Sex and ancestry estimation from landmarks of the cranial base. *Proceedings of the American Academy of Forensic Sciences*; Seattle, WA., 2010;16:375.

Cranial Base, Ancestry, Identification

H43 A Test of the Megyesi Equation on Scavenged Human Remains

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After attending this presentation, attendees will be better able to conceptualize the importance of temperature in the decomposition process and to understand the limited role small scavengers may play.

This presentation will impact the forensic science community by contributing to an understanding of human decomposition so that the accuracy of postmortem interval estimates can be improved.

The purpose of this research is to address the contribution of small vertebrate scavengers to human decomposition and to discuss how such scavenging may affect estimates of postmortem interval calculated using the regression equation proposed by Megyesi *et al*.[1] Megyesi's equation uses a scoring scheme to produce a numerical representation of the state of decomposition and can be used to predict the number of accumulated degree days that have elapsed since death. Weather data

can then be used to relate accumulated degree days to chronological time since death. Postmortem interval estimates derived from Megyesi's equation assume that the cadaver is that of a complete adult human that has not suffered any trauma and has been allowed to decompose on the surface. Megyesi et al.call for future studies to address whether the equation accurately predicts postmortem interval in the event that the remains deviate from these specifications. Scavenging is one such deviation and is addressed in this project.

The scoring scheme described by Megyesi *et al*., the total body score, was used to document decomposition over a period of months for six donated human cadavers placed at an outdoor human decomposition research facility in western North Carolina. Motion sensitive cameras were used to gather evidence of scavenging. All six donated cadavers were scavenged by small mammalian and avian species.

Actual accumulated degree days were calculated for each donor and plotted against total body score as reported by observers. The resulting pattern for the entire data set was curvilinear, with an R-squared value of 0.395. Accumulated degree day values were transformed using a base-10 logarithm, and the resulting plot for the entire data set was more linear with an R-squared value of 0.75, suggesting that, when transformed, accumulated degree days are a strong predictor of variation in total body score.

When the regression equation of Megyesi et al. is applied to this data set, the equation worked reasonably well to predict the actual accumulated degree days when the suggested error range of +/-776.32 accumulated degree days was applied. However, a paired sample T-test of the actual accumulated degree days against the predicted accumulated degree days showed that the differences in the means were significant at $p<0.001$. Autocorrelation may be a factor because scores are from sequential observations of the same individuals. These results indicate that Megyesi's findings of the strong correlation between thermal energy, as measured by accumulated degree days, and level of decomposition hold true even in the presence of scavenging and in the western North Carolina region. However, future research in this biome may provide a refined regression equation for similar environments and may serve to quantify the contribution of scavenging to the percentage of total body score that is not explained by accumulated degree days.

Reference:

1. Megyesi MS, Nawrocki SP, Haskell NH. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. J Forensic Sci 2005;(50)3: 618-26.

Decomposition, Postmortem Interval, Scavenging

H44 Estimation Using Postcranial Measurements: A Validation Study of Spradley and Jantz on a White Population

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After attending this presentation, attendees will better understand the results and implications of a recent test of Spradley's and Jantz's postcranial classification rates for sex determination using two North American White collections.

This presentation will impact the forensic science community by demonstrating the accuracy of select univariate and multivariate postcranial measurements when determining sex on a large, known White sample.

A recent publication by Spradley and Jantz tested the accuracy of various discriminant functions using cranial and postcranial skeletal measurements from the Forensic Databank.[1] The purpose of the current study was to verify these univariate and multivariate postcranial formulas on individuals from two regionally diverse skeletal collections.

Thirty-five postcranial measurements were taken for 139 individuals from two documented skeletal collections in Canada and the U.S. Fifty-nine individuals (24 females and 35 males) were measured from the J.C.B. Grant Collection housed at the University of Toronto, and 80 individuals (35 female and 45 males) were measured from the collection housed at the Maxwell Museum in Albuquerque, New Mexico. Individuals in this study were between the ages of 18 and 101 years. As these collections are primarily composed of White adult individuals, only this ancestry group was tested in this study. Each collection was measured independently by the authors, and intra-observer tests were conducted on randomly selected sub-samples of the individuals used in this study.

Since most of the crania available were previously sectioned, only postcranial measurements could be tested. In total, 35 postcranial measurements which yielded accuracy rates of 80% or higher in the original study were used. Sectioning points were calculated in the same manner as that used by Spradley and Jantz. Once the sectioning points for each univariate and multivariate function were gathered, they were ranked in descending order and compared to the hierarchies published in the original study for the American White population.

The current study found that overall, the classification rates for both univariate and multivariate sex estimation functions are consistent with those reported by Spradley and Jantz. Of particular note are the accuracy rates achieved by the univariate humeral epicondylar breadth and the multivariate function for the scapula. Both of these functions yielded accuracy rates of 92% or above in both collections, suggesting these measurements are reliably applied to a wider sample of White individuals. Conversely, despite the fact that the univariate calcaneal accuracy rates are high between both studies' samples (Spradley and Jantz, 76% for calcaneal length and breadth; Maxwell sample, 82% and 74%, Grant sample, 60% and 71% for calcaneal length and breadth, respectively), the accuracy rate yielded by the original study for the multivariate calcaneal function (length and breadth) was nearly twice that of the validation sample's (Spradley and Jantz, 83%; Maxwell sample, 41%; and Grant sample, 44%); suggesting that an error may be associated with the original multivariate function.

The results of the current study strongly support and validate the claim set forth by the Spradley and Jantz equations, and shows that postcranial measurements can be reliably used to estimate the sex of unidentified individuals. Given these results, this paper hopes to justify a more widespread adoption of postcranial sectioning points for sex estimation within the forensic anthropology community.

Reference:

1. Spradley MK, Jantz R. Sex estimation in forensic anthropology: skull versus postcranial elements. *J Forensic Sci* 2011;56(2): 289–96.

Sex Estimation, Postcranial Elements, Sectioning Points

H45 Stature Estimation From Long Bone Lengths Among the Adult Colombian Population

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After attending this presentation, attendees will be made aware of a new modern Colombian skeletal collection from which population-specific stature formulas for adult Colombian males and females are recently derived. The educational objectives are to present these new formulas and to compare the accuracy of these new formulas compared to previously published formulas.[1,2]

This presentation will impact the forensic science community by helping to develop population standards in Colombia that could potentially play a role in the identification of thousands of unidentified human remains from the ongoing conflicts in Colombia.

The aim of this study is to develop general formulas for stature estimation using inverse regression of long bone lengths (humerus,

radius, ulna, femur, tibia, and fibula) of Colombian adult males and females. Previous studies to estimate stature of individuals from the Colombian population made by Mantilla Hernández (2009) using tabias demonstrate that these population-specific formulas are more precise than those developed by Trotter and Gleser and Genovés.[1-3] This new collection will provide modern population-specific equations using a known modern skeletal collection to improve the accuracy of stature estimation. For this study, a new skeletal sample is used called the Human Skeleton Collection of Colombia, which is curated at the Institute of Legal Medicine and Forensic Sciences (INML y CF) and was developed under the supervision of Dr. César Sanabria. The Human Skeleton Collection of Colombia consists of individuals of known age, sex, and stature (stature from ID cards and/or cadaver stature) and offers a unique opportunity to develop and test stature formulas specific to the Colombian population. The individuals have dates of death from 2005. The study was conducted on 140 skeletons, 99 males and 41 females, aged 19 years and above, with an average age of 47 years. The collection and this research project have been part of a bigger collaboration to develop standards from this new sample with the help of the International Criminal Investigative Training and Assistance Program (ICITAP). By convention, the left bones in each skeleton were measured. Nine parameters (maximum lengths of humerus, radius, ulna, femur, tibia (without the intercondyloid eminence), and fibula)) were recorded. The current study uses measurement of the tibia that includes the malleolus, which has become standard for stature estimation. For the statistical analysis, linear and multivariate regression equations were generated for all bones for each sex separately. Only regressions with highly significant F values ($p<0.01$) were accepted. All of the long bones of the upper and lower limbs are included in univariate inverse regression formulas and the best multivariate regression equations are calculated using a stepwise procedure. All of the equations are significant for males and females ($p<0.01$). The equation from the femur for males (Stature=(Femur*2.46) + 5.9004 +/- 3.760) has a high correlation (r=0.857). The equation from the femur for females (Stature=(Femur*1.787) + 83.592 +/- 4.951) has a much lower correlation (r=0.692). The best multivariate equation for males was from the femur and tibia combined (Stature=(Tibia*1.592) + (Femur*0.967) +67.348 +/- 3.091; r = 0.891, adjusted R^2 =0.784). The best equation for the females was a univariate equation from the fibula (Stature=(Fibula*2.183) + 93.170 +/- 5.065; r=0.697; adjusted R^2=0.460). These results indicate that the sample size for the males is sufficiently large for reliable stature equations, but the sample size for the females should be larger to improve the correlations for reliability in forensic stature estimation. Since this study was first conducted, the Human Skeleton Collection of Colombia sample has since grown in size to be several hundred individuals, which will enable improved equations of females and the ability to test the validity of the male equations on an independent sample.

References:

1. Mantilla Hernández JC, Cárdenas Durán N, Jácome Bohórquez M. Estimation of height from measurements of the tibia in Columbian population. *Int J Morphol* 2009;27(2):305-309.
2. Trotter M, Gleser G. A re-evaluation of estimation of stature based on measurements of stature taken during life and of long bones after death. *Am J Phys Anthropol* 1958;16:79-123.
3. Genoves S. Proportionality of the long bones and their relation to stature among Mesoamericans. *Am J Phys Anthropol* 1967;26: 67-77.

Stature Estimation, Femur, Tibia

H46 Asymmetry of the Humerus: The Influence of Handedness on the Deltoid Tuberosity and Possible Implications for Osteometric Sorting

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After attending this presentation, attendees will have gained an understanding of how individual asymmetry may affect the sorting of humeri in a commingled assemblage and the possible correlation between this asymmetry and an individual's handedness.

This presentation will impact the forensic science community by illustrating which measurements may be most related to hand preference and whether this could adversely affect the osteometric sorting method for pair-matching.

The identification of individuals from commingled human remains can be a difficult task. Osteometric sorting is often utilized for these purposes since it sorts remains based on size. This research investigates whether asymmetry of the humerus is present, if it is due to an individual's hand preference, and if this asymmetry can cause incorrect sorting of elements when utilizing the osteometric sorting method for pair-matching.

The osteometric sorting formulas for pair-matching were used to classify individuals as asymmetric. This method utilizes a *p*-value to determine whether a significant difference in size between left and right elements exists. A *p*-value less than 0.10 indicated that two elements were significantly different, and therefore, deemed to be asymmetrical. Significant asymmetry was seen in multiple measurements and this asymmetry could be due to handedness. This relationship appeared more consistent with breadth measurements than length measurements, and the right side appeared to be larger more often. The epicondylar breadth of the humerus was the most asymmetric with 12.8% of individuals considered asymmetric. All breadth-only measurements of the humerus resulted in over 12% asymmetric. Asymmetry of the maximum length of the humerus occurred in only 7.3% of individuals. When breadth measurements were combined with the maximum length of the humerus, the number of the asymmetrical individuals was reduced to less than 9%. These results did not appear to correlate with the results seen on the radius. Maximum length of the radius was slightly more asymmetric than the maximum diameter of the radial tuberosity, 9.9% and 8.3%, respectively. Less than 20% were considered asymmetrical for both the radius and humerus. This indicates that asymmetry in the arm and forearm is not related.

The femur yielded results indicating asymmetry between left and right, but these results were not significant (only 6% of individuals). The mean difference of the maximum length of the femur between left and right elements was 1mm, which is incredibly small for a measurement that is generally at least 400mm.

When investigating male and female differences of the humerus, a notable difference was found. The range of the difference between left and right elements was greater for males than for females for both maximum length of the humerus (28.00mm and 11.50mm) and the maximum diameter at the deltoid tuberosity (7.6 mm and 2.67mm). The osteometric sorting method uses the variation in the data set to determine whether the difference between two paired elements is significant or not. When females are included in the data set with males, the difference between paired elements is not significant, but when analyzed alone, they are. This suggests that, in general, males have a greater difference between left and right humeri than females.

Asymmetry is present in the upper limbs. A significant difference in size between the left and right humeri and radii was seen. However, particular measurements were more asymmetrical than others. Breadth measurements were asymmetrical more often than length measurements. This asymmetry may be due to an individual's hand preference. Further investigation is necessary. No correlation was found between asymmetry of the humerus and asymmetry of the radius. This suggests that mechanical forces may act differently on the upper limb than the lower limb. These results indicate that anthropologists

utilizing the osteometric sorting method should be aware of measurements that tend to reflect asymmetry.

Asymmetry, Humerus, Osteometric Sorting

H47 The Potential for Sex Assessment From Dental Dimensions in Modern Forensic Cases

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The goal of this presentation is to examine the degree of sexual dimorphism in tooth dimensions and to explore the utility of these measurements in the assessment of sex.

This presentation will impact the forensic science community by investigating the potential of a method of sex assessment that could be used in cases where other indicators of sex are absent or ambiguous.

One of the most crucial steps in constructing the biological profile of an unknown individual is the assessment of biological sex from skeletal remains. Estimation of other aspects of the biological profile often relies on a prior knowledge of an individual's sex. However, in many forensic cases, the most diagnostic elements for sex estimation may not be present, have been damaged, or provide ambiguous results. Additionally, morphological and metric methods for sex assessment in subadult skeletal remains have not been reliably established; however, the permanent dentition has been used to accurately sex subadults with mixed dentition. Since 1972 at the C.A. Pound Human Identification Laboratory, approximately 20% of cases involved examination of either subadult remains or the skull/cranium or mandible as the only element available for sex assessment. In cases such as these, other means for assessment of sex from skeletal remains need to be investigated.

Human dentition is highly controlled by genetics and is the most durable portion of the human skeleton. Previous studies have shown that the adult dentition is sexually dimorphic, with sex prediction accuracies ranging from 62.3% to 85%. However, many of these studies are restricted primarily to archaeological populations or casts from living individuals. In forensic cases, odontometrics may prove a feasible option for sex assessment when remains are fragmentary, ambiguous, or a subadult with mixed dentition.

The current study examines the potential utility of odontometrics for sex assessment in a contemporary European American sample of forensic cases analyzed and curated at the C.A. Pound Human Identification Laboratory. Digital extended pointed jaw calipers were used to measure the mesiodistal and buccolingual diameters of the permanent dentition of 33 males and 20 females (total *n*=53). Measurements were not taken in dentition where attrition, carious lesions, restorations, or dental crowding prevented an accurate measurement. Due to these conditions, as well as antemortem and postmortem tooth loss, not all teeth are equally represented within the sample; however, this reflects actual conditions of remains in forensic casework. Student's *t*-tests were used to determine which dimensions were statistically different between the sexes, and sectioning points were calculated from these dimensions. Measurements higher than the sectioning point are classified as male, and those smaller than the sectioning point are classified as female.

Paired *t*-tests revealed statistically significant differences between the sexes (at a 0.05 alpha-level) in seven buccolingual dimensions: LI^2, LC′, LP^4, LI_2, LC′, LM_1, and RC_1 and four mesiodistal dimensions: LC^1, LP^4, RM^1, and LC_1. Sectioning points were established using a weighted mean for each of these teeth. Additionally, a discriminate function analysis was performed using those teeth that exhibited statistically significant differences in size between the sexes. Within this subset of the dentition, the combined buccolingual dimensions of LI^2 and LC^1 provide the highest accuracy levels at 77% for cross-validated correct classification of both males and females within the sample. Interestingly, the buccolingual dimensions are often easier to take than the mesiodistal measurements, particularly in modern populations, which exhibit significant dental crowding.

The correct classification of 77% falls within the range of accuracy levels of previous sexual dimorphism studies from the dentition. However, due to the established population specificity of sexual dimorphism in teeth, these results may not be applicable to non-European ancestral groups. Regardless, this study suggests that dental dimensions may provide additional information that should be considered in the assessment of sex, and may greatly aid in the analysis of fragmentary, incomplete, or subadult remains.

Sex Assessment, Odontometrics, Forensic Anthropology

H48 Pelvic Sexual Dimorphism in a Western Australian Population: Integration of Geometric and Traditional Morphometric Approaches

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After attending this presentation, attendees will have an understanding of the utility of geometric and traditional morphometric approaches for the quantification of sexual dimorphism in the adult pelvis and how both approaches offer different (albeit complimentary) insights.

This presentation will impact the forensic science community by demonstrating the value of medical scans as alternate sources of contemporary population-specific skeletal data. The presentation also demonstrates that in the Western Australian population, it is possible to estimate sex with a high degree of expected accuracy using traditional pelvic measurements.

In the analysis of unidentified skeletal remains, it is crucial to formulate an accurate biological profile. The correct assignation of sex to skeletal remains not only contributes to establishing personal identity, but also provides an essential criterion for ensuring that the appropriate sex-specific age, ancestry, and stature standards are subsequently applied. It is widely accepted that the most accurate biological profile is achieved through the application of contemporary population-specific standards. The forensic anthropological community in Western Australia (WA) is currently working toward developing a suite of population-specific standards. Furthermore, in the absence of reference skeletal material (of a contemporary population and in appropriate numbers) a morphometric approach involving the analysis of Multi-Slice Computed Tomography (MSCT) scans has been adopted. This presentation demonstrates the sexual dimorphism of the pelvis in a contemporary WA population. This is achieved using both a 3D shape and traditional linear measurement approach. The study is part of ongoing research in population-specific standards from MSCT scans of living individuals.

The sample comprises pelvic MSCT scans with a mean slice thickness of 1.2 millimeters from 50 male and 50 female adults with a mean age of 46.94 years (range 22 – 63) for males and 45.76 years (range 21 – 62) for females. Following 3D volume rendering, the 3D coordinates of 41 landmarks were acquired using OsiriX® (v.4.1.1). A total of 30 linear measurements, suitable for a complete and/or a fragmented bone, were calculated using Morph Db (an in-house developed database application). Measurements were analyzed using basic descriptive statistics and discriminant function analyses; statistical analyses are performed using IBM® SPSS® Statistics 20.0. Shape analysis software morphologika (v.2.5) analyzed the 3D coordinates of the landmarks. Principal Components (PCA) and multivariate

regression analyses were used to explore relationships between the male and female samples. Shape differences were visualized and interpreted using 3D wireframe and rendered models. Significance of sexual dimorphism in pelvic shape was quantified using permutation tests for mean differences, whereby the true difference between means (Procrustes distance) is compared with the distribution of differences between means obtained by randomly permuting group membership 1,000 times.

Multivariate regression (PCs 1-5; 53.5% total variance) and permutation tests indicate significant pelvic sexual dimorphism in the WA population; males (relative to females) demonstrate an acute sub-pubic angle, narrow and short pelvic inlet/outlet, long and narrow ilium, and an anteriorly curved sacrum. A total of 24/30 linear interlandmark measurements are sexually dimorphic and sex differences explain 3.7% – 68% of sample variance. Cross-validated sex classification accuracy (stepwise and direct DFA of linear measurements) for the complete pelvis is 96%; measurements for a complete os coxa yielded accuracy rates above 90%. Other dimorphic regions in the pelvis, which may be useful for assessing fragmentary remains, include (but are not limited to) the ischium (ischial length: 88% accuracy) and the acetabulum (length and width: 84% accuracy). Only those discriminant functions with an accuracy ≥80% with a sex bias of ≤5% are deemed suitable for forensic application.

This preliminary study represents the initial forensic research into pelvic sexual dimorphism in a WA population. It was clearly established that this bone can be used to classify sex with a high degree of expected accuracy, irrespective of whether a complete or fragmented bone is examined. Future research will concentrate on expanding the pelvic database and further testing the derived standards using hold-out samples and resampling statistics, thereby improving the statistical robustness of the formulated standards and demonstrating their ability to meet the *Daubert* admissibility requirements.

Sex Estimation, Pelvis, MSCT

H49 Metric vs. Non-Metric: Accuracy in Sex Assessment Using the Greater Sciatic Notch

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After attending this presentation, attendees will understand the effects of chosen methodology on sex assessment of the human pelvis. This presentation provides an analysis of whether metric or non-metric analysis of the Greater Sciatic Notch (GSN) provides greater accuracy in determining sex. Attendees will observe the results of comparisons between the predicted sex through non-metric assessment following Buikstra and Ubelaker and metric evaluation using the GSN index.[1]

This presentation will impact the forensic science community by explaining how, ideally, multiple morphological features are used in assessing sex in skeletonized remains. However, in forensic contexts this is not always possible. These results highlight that neither the traditional visual assessment of sex using the GSN nor the metric method employing the GSN ratio is perfect. Nonetheless, the combination of both methods generates the greatest accuracy in this sample. The importance of these results is in highlighting the accuracy of varying methods of assessing sex through the GSN of the os coxa. This area is robust and frequently survives both archaeological and forensic contexts. The results of this research provide an invaluable addition to physical anthropology with practical applications for archaeological analysis and forensic casework.

Human skeletal pelvic dimorphism, specifically the sciatic notch, has long been a topic of discussion in physical and forensic anthropology. Historically, the "tools of the trade" for assessing sex in the pelvis have focused on visual, nonmetric methods, which are often conducted without an ecogeographically specific standard for comparison. In addition, nonmetric methods make use of relatively ambiguous descriptors for assessment including "broad," "comparatively open," and "shallow" for females and "narrow," "deep," or "J-shaped" to indicate male. As for metric analysis, Walker notes that "although many attempts have been made to describe sex differences in the sciatic notch using measurements, these metrical sexing techniques have not been widely adopted."[2] Despite Walker's comments, many researchers have repeatedly found that metric analysis of the pelvis, specifically the sciatic notch, provides a greater and more accurate sex assessment. This investigation empirically tests these contrasting hypotheses.

The population examined included segments of the Terry Black (n=99) anatomical collection (all remains included in this analysis are housed at the National Museum of Natural History, Smithsonian Institution). Individuals were sampled from both sexes: 50 female, 49 male. All nonmetric and metric evaluations for the sex of each individual were completed by the first author. Metric assessment was made through measurement of the GSN index; the ratio of the posterior chord of the GSN divided by the maximum width of the GSN. Nonmetric assessment was evaluated following Buikstra's and Ubelaker's five-stage system generally employed in physical and forensic anthropology.[1] It was then determined whether metric and nonmetric assessments agreed or were in conflict. These results were compared to the known sex of the individual to determine whether there was agreement between the methods and if not, which method is more accurate.

The present research found that 77% of the sample was assigned the correct sex using the nonmetric method alone and 81% of the sample correctly sexed through the metric method employing the GSN ratio; however, when the two methods were combined, 92% of the total sample was sexed correctly.

Ideally, multiple morphological features are used in assessing sex in skeletonized remains; however, in forensic contexts this is not always possible. These results highlight that neither the traditional visual assessment of sex using the GSN nor the metric method employing the GSN ratio is perfect. Nonetheless, the combination of both methods generates the greatest accuracy in this sample. The importance of these results is in highlighting the accuracy of varying methods of assessing sex through the GSN of the os coxa. This area is robust and frequently survives both archaeological and forensic contexts. The results of this research provide an invaluable addition to physical anthropology with practical applications for archaeological analysis and forensic casework.

Refereneces:

1. Buikstra JE, Ubelaker DH, editors. Standards for data collection from human skeletal remains: proceedings of a seminar at the Field Museum of Natural History. Fayetteville: Arkansas Archaeological Survey Research Series No. 44, 1994.
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Sciatic Notch, Sex Assessment, Metric Analysis

H50 Chin Form as a Sex Trait: Not a Simple Gradient

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After attending this presentation, attendees will have a better understanding of the morphological variation and sexual dimorphism in chin form present across diverse human populations, and the reliability of skeletal chin traits in adult sex determination methods.

This presentation will impact the forensic science community by providing sex classification rates for overall chin morphologies. In comparison to previous studies, which utilized subjective ordinal scaling methods, this study uses an objective morphometric method to quantitatively analyze sex differences in chin shape.

Chin form is a common non-metric cranial trait used by forensic anthropologists in adult sex determination methods. Traditional sex estimation methods present an ordinal scale of five line drawings, representing a gradient in chin form from more "feminine" to more

"masculine" morphologies.[1] Because these observed sex differences are in the form of localized shape changes and complex variations in bony relief, finding a method to quantify sex differences in external chin morphology has presented a great challenge to anthropologists. A recent study published a method to isolate the chin Region Of Interest (ROI) from 3D surface scans, and demonstrated how morphometric methods could then be used to quantify sexual dimorphism in chin morphology.[2]

This study employs their method of isolation and uses sliding semi-landmarks positioned across the surface of the chin ROI to quantify chin morphology in order to objectively test the reliability of external chin form in discriminating between sexes. The accuracy of using quantitative measurements of chin form to correctly sex individuals was evaluated on a pooled sample (n=666), as well as separately on each of the six population groups included in this study: U.S. Whites, U.S. Blacks, Arctic Native Americans, Plains Native Americans, Nubian, and Portuguese. Discriminant function analyses, using a leave-one-out cross-validation method, were performed on the principal components extracted from the morphometric analyses. Traditional ordinal scores were also collected on four of the population groups, and discriminant function analyses were performed on these ordinal scores for comparison to the morphometric results.

Overall, results suggest that external chin morphologies are not reliable indicators of sex and consequently, should be used with extreme caution in forensic anthropological contexts. Discriminant function analyses run on the first 30 principal components, reflecting overall chin morphology, resulted in a correct sex classification rate of 68.2% for the pooled sample. Within individual population groups, accuracy rates ranged from 49.4% for the Nubian population to 70.4% for the arctic Native American population. Only a 50.8% correct classification rate was obtained for the U.S. White population group, while U.S. Blacks displayed slightly higher accuracy rates (65.0%). These results were on par with the accuracy rates obtained from the traditional ordinal scores (65.0% correctly sexed in the pooled sample). Step-wise discriminant function analyses suggest that the shape component reflecting relative chin ROI height compared to breadth was the most sexually diagnostic feature, with males generally displaying relatively taller chin symphyseal heights than females. Principal component plots illustrate a high degree of variance and overlap between sexes and population groups in chin morphologies. In addition, individual chin features, such as degree of "squareness," symphyseal height, overall chin protrusion, midline eminences, and lateral tubercle formations, were observed in various combinations across the sample. These results suggest that specific chin traits may be expressed independently, and that chin form may not be easily categorized into a simple gradient from more "feminine" to more "masculine" forms. The existence of numerous distinct categories of chin morphologies was first presented in the 1930s, but has since been neglected in the anthropological literature.[3,4]

As demonstrated in this study, the external morphology of the chin is much more variable than traditional non-metric studies suggest. Oversimplified gradients may not accurately reflect complete variation in observed chin forms, and the independent expression of individual chin traits should be further investigated. Correct sex classification rates obtained from chin shape components did not exceed 70%, and in two population groups less than 51% of the individuals were correctly sexed from chin morphologies. These results indicate that chin form is not a reliable indicator of sex, and thus is not recommended for use in forensic sex determination methods.

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Chin, Mental Eminence, Sex Determination

H51 Sexual Dimorphism of Immature Pubis Bone: A Multislice Computed Tomography Study by Geometric Morphometrics

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The goal of this presentation is to study pubis morphology during ontogeny, specifically examining size and shape dimorphism independently.

This presentation will impact the forensic science community by: (1) demonstrating pubis ontogeny in order to develop new methodology for sexing pubis bone in juveniles; (2) showing the potential of Three-Dimensional (3D) Multislice Computed Tomodensitometry (MSCT) reconstructions for future anthropological research; and, (3) showing the potential of using clinical MSCT investigations for anthropological purposes, rendering the concept of virtual anthropology more concrete.

Purpose: Recent developments in geometric morphometrics open new research avenues in biological anthropology. Difficulty in sex estimation of juveniles is frequently encountered in forensic medicine. Contrary to adult remains, few studies have been conducted to evaluate sex differences of immature coxal bone and, when studied, the results are often contradictory. The objectives of this study are to analyze the ontogeny of size and shape sexual dimorphism in the pubis.

Materials and Methods: A retrospective study to evaluate sexual dimorphism observed in the pubis was conducted. A combination of landmark-based geometric morphometric analyses using 3D reconstructions of MSCT were performed to evaluate the ontogeny of pelvic shape and size dimorphism. Specifically, the analyses were designed to examine differences between age groups and rates of change. Five osteometric landmarks were targeted. The study population consisted of 188 children (95 boys, 93 girls), ranging in age from 1 to 18 years. The subjects were of diverse ancestry and lived in the area of Toulouse, southern France. Data were collected by two observers following a data collection protocol. Briefly, PCA was used as an exploratory step. Two-way MANOVA and Goodall's F-test were used to test differences between the sexes and among age groups. Linear regression was used to compare rates of shape change. The authors analyzed sexual dimorphism in size (centroid size) and shape (Procrustes residuals) and patterns of shape change with age (development) and size change with age (growth). MorphoJ and R2.2.0 software was used to perform the statistical tests.

Results: The maximum intra- and inter-observer error was 3%. The results of the PCA analysis demonstrated that the ontogenetic trajectories of shape change were different for males and females. Two-way MANOVAs of shape variables, with sex and age as factors, revealed statistically significant differences ($p<0.001$) between the sexes and age groups. The pubis shape became significantly sexually dimorphic at 13-years-old, although visible shape differences were observed as early as 9-years-old. Also, the size was statistically significant between the sexes. Trajectories of shape (development) and size (growth) differed between sexes throughout ontogeny. Mean shape

superimpositions between sexes for each age group showed that shape differences between sexes occurred gradually with age.

Conclusion: Immature pubis bone sexual dimorphism is an age-dependent phenomenon, both manifesting by size and shape differences. The immature pubis presents sexual dimorphism in both size and shape. In ontogeny, pubis growth and pubis development are separate phenomena.

Geometric Morphometric, Ontogeny, Pubis

H52 Postcranial Osteometric Analysis of Korean Ancestry

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After attending this presentation, attendees will have an understanding of a new method of ancestral classification that can be utilized when only postcranial remains are available as well as the regions of the skeleton yielding the greatest predictive ancestral accuracy when using osteometric sorting measurements and standard maximum length measurements.

This presentation will impact the forensic science community by providing information on a new postcranial method for the determination of ancestry and by providing forensic anthropologists an assessment which can be applied to incomplete or fragmentary remains, thereby increasing the possibility of identification.

Determination of ancestry is an important step in the development of the biological profile, which is made more challenging when the skull is not present for analysis. Previous postcranial research has focused heavily on differentiating Europeans and Africans from one another. When an Asian skeleton is present in ancestry analyses, the sample population is frequently limited to Native Americans. This research examines the potential to correctly predict ancestral classification of remains using an Asian sample from South Korea and African, and European samples from four collections in the United States.

Osteometric sorting measurements were originally developed to assist anthropologists in sorting commingled remains. Measurements focus on morphological landmarks, minimum and maximum diameters, and minimum and maximum breadths making it so complete bones are not necessary for assessment and increasing the possible information that can be utilized from fragmentary remains. ANOVA tests were used to select measurements displaying significant differences between the three ancestral populations, and these measurements were then grouped according to their corresponding skeletal region (upper limb, hand, pelvis, lower limb, and foot). Forward stepwise discriminant function analysis then minimized the number of measurements utilized in each of the resulting functions to only the most significant. To prevent inflated classification results discovered in preliminary testing, sample populations were assessed in a two-part analysis: Koreans from Africans/Europeans and Africans from Europeans. All functions contain between one and four measurements.

Of the 14 functions synthesized to differentiate Koreans from Africans/Europeans, most had cross-validated correct classification rates of 80% or greater for Koreans and 77% or greater for the pooled African/European sample. The highest classifying function for separating both groups consisted of upper limb measurements. Of the postcranial skeletal elements measured, the clavicle, ulna, and femur were most frequently selected in analyses to distinguish Koreans from Africans/Europeans. When separating Africans from Europeans, the lower limb measurements were found to be too similar for them to be differentiated using stepwise discriminant function analysis, so only 13 functions were produced. Most of the functions when cross-validated, correctly classified Africans with 70% or greater accuracy and Europeans with 72% or greater accuracy. The best discriminating function with the highest classification rates for both Africans and Europeans consists of a combination of pelvis, lower limb, and foot measurements. Elements included in the most functions for these two ancestral groups were the sacrum, cuboid, and radius.

Independent discriminant analyses also scrutinized differences in limb proportion between Koreans and Africans/Europeans as well as Africans from Europeans through standard maximum length measurements. Analysis of the humerus, radius, and ulna separated Koreans from Africans/Europeans with a cross-validated overall accuracy of 79.8%. Maximum length of the femur and tibia had a cross-validated correct classification rate of 81.9%, while the femur alone had a cross-validated correct classification rate of 82.1%. Comparison of maximum lengths of the humerus and femur differentiated with an overall cross-validated correct classification rate of 80.9%. Maximum lengths of the clavicle, humerus, radius, and ulna were able to differentiate Africans from Europeans with a cross-validated overall accuracy of 81%. Analyses of lower limb lengths were less discriminating, but when combined with those of the upper limb, overall classification accuracy (cross-validated) was 75.6%.

Ancestry can be determined with acceptable accuracy from complete and fragmentary remains using osteometric sorting measurements. Similarities in size of Africans and Europeans make it more advantageous to determine Asian from non-Asian ancestry first, instead of attempting to predict group membership against all three groups. The skeletal elements found to be the most conducive to each of the two analyses differ due to proportional as well as size differences. Further analyses are necessary to determine the potential influence sex may have on ancestral classification.

Postcranial, Osteometrics, Ancestry

H53 Application of Cranial Indices to Estimate Ancestry in Modern and Historic South Africans

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After attending this presentation, attendees will gain knowledge of the applicability and accuracy of using cranial indices to estimate ancestry in modern Black and White South Africans and the historic Khoisan.

This presentation will impact the forensic science community by contributing to the knowledge of human variation in South Africa through testing the validity of current methodology. The results are to be used to establish best practices for estimating ancestry among modern South Africans.

In a diverse country like South Africa, the estimation of ancestry is an essential component for both the biological profile and the identification process. De Villiers and Steyn employed five cranial indices to quantify morphological differences among Black and White South Africans and indigenous Khoisan groups.[1-2] Despite the absence of validity testing and the advent of robust statistical analyses, the mean values for these cranial indices continue to be used to separate these groups and to classify skeletal remains as either modern or archaeological.

The purpose of this study was to establish the accuracy of five standard cranial indices, namely the Cranial Index (CI), Upper facial Index (UI), Orbital Index (OI), Nasal Index (NI), and Gnathic Index (GI), in differentiating Black/White South Africans and Khoisan groups.

A total of 207 crania (110 females, 97 males) of Black South Africans, White South Africans, and Khoisan were used. White and Black groups were obtained from the Pretoria Bone Collection in South Africa, while skeletal remains of known Khoisan origin were obtained from the Rudolph Pöch Collection in Austria. Ten standard measurements were taken using a spreading caliper and a digital sliding caliper. Five indices were calculated and compared to specific

sectioning points that had been assigned to the above-mentioned groups in *Fisiese Antropologie*.[2] Statistical analyses included an ANOVA and Tukey's method to test mean comparisons and statistical significance and percent correct to test accuracy of the indices.

For all five indices, two or more of the three groups had similar mean values. All indices had similar means for Black and Khoisan groups and no statistically significant differences were noted between them. With UI, no statistically significant differences were found among the groups. White and non-White groups demonstrated statistically significant differences for CI, GI, and NI. With OI, Khoisan groups demonstrated statistically significant differences from Black and White groups, but the latter two groups demonstrated no statistically significant differences between each other for this index.

For the groups that demonstrated statistical significance, new sectioning points were both created manually and using the Fisher-Jenks test. When the new and original sectioning points were compared, accuracy rates were generally higher for the new sectioning points (CI=73.55%; OI=71.57%; NI=88.18%; GI=79.88%) than the original sectioning points (CI=34.9%; OI=78.85%; NI=90.63%; GI=48.09%). Due to similar means, the sectioning points are usually only distinguishing White from non-White groups.

With the current sectioning points and group separation, cranial indices are not useful for describing differences among these social labeled groups. While Khoisan is a strong historical term, the crania of this group are generally not morphologically distinct from Black South Africans. Thus, the social designation is neither useful to understand ancestry in the population nor to sort modern and archaeological remains. Similar to other studies, White South Africans are clearly distinct from their non-White counterparts.[3,4] Possible reasons include later emigration of White groups into South Africa (1652); past segregation laws; and social/cultural behavior.[4] While the average accuracy of cranial indices is not as high as other more statistical robust methods, a revision of cranial indices and of their correction points has made this a valid and more appropriate method for use in South Africa.

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Human Variation, Sectioning Points, Best Practice

H54 Fluvial Transport Distances and Postmortem Interval in the Sacramento River, California

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After attending this presentation, attendees will gain a better understanding of the relationship between fluvial transport distance and Postmortem Interval (PMI) for human remains recovered from the Sacramento River, California. The goal of this presentation is to evaluate the key variables that influence transport rates of human remains in fluvial systems.

This presentation will impact the forensic science community by promoting an understanding of how river systems transport human remains and how this information can be used to establish a predictive model for narrowing down search parameters for victims who entered the river on known dates.

The transport of human remains in riverine systems has received attention by forensic anthropologists and law enforcement over the past several decades, with effort directed at search areas for missing persons. Until recently, studies of fluvial transport of human remains have largely been limited to the eastern United States.[1-3] However, recent research conducted within the Sacramento River found that long-distance transport is correlated with high discharge rates at the time the victim entered the river.[4] This research hypothesized a correlation between discharge rates and transport distances over known PMIs. The present study examines this relationship using a large sample of river victim cases.

The Sacramento River comprises the largest fluvial system in California, flowing 335 miles north-to-south, and passing though eight counties in northern California. Discharge rates vary based on annual snowmelt and precipitation, with an average rate of 350m^3/sec. Modeled after previous research, this project involves two broad categories of variables: victim demographics and river dynamics.[3] Demographic variables include the biological profile of the victim; victim weight; PMI, or number of days in the river; date of river entry and exit; location and side of entry and exit; preservation state; manner and cause of death; and the number of river miles traveled. The study sample currently includes data on over 120 cases from sheriff's/coroner's offices in three northern California counties. These cases span the 1970s to present and include all cases where the PMI exceeded two hours. Variables related to river dynamics include: season; water and air temperature; water discharge rate; depth; and bed load. Transport rates of human remains were estimated using recorded average United States Geological Survey (USGS) water discharge rate records between the nearest river mile data collection station to each victim's point of entry and exit.

Together, variables from these two categories have demonstrated a relationship with transport rates of human remains within this fluvial system. Analysis of the current data indicates that the majority of victims are found within several hours to several days of entering the river, and they are usually found within two miles of the location of entry. Under these circumstances, water discharge rates do not factor significantly in the fluvial transport of human remains; however, for PMIs longer than several days, hydrological factors can significantly influence transport. In these cases, transport distances are associated with rapid increases in water volume, especially in cases in which dam regulation is used to offset heavy rainfall or snowmelt into the river.

While results indicate that the majority of individuals are more likely to be found in close proximity to where they entered the river, the addition of known death and recovery dates from case files from additional counties along the river, in conjunction with river discharge rates, will permit more precise estimations of transport rates for different sections of the river. This research will help to narrow down search parameters for locating victims.

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Taphonomy, Fluvial Transport, Postmortem Interval

H55 Skeletal Trauma in the Tuskulenai Case: A Comparison of State-Sponsored Violence in the Former Soviet Union

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After attending this presentation, attendees will gain an understanding of how skeletal trauma varies between executioners in the Tuskulenai case and how state-sponsored violence in the former Soviet Union varied throughout the Stalin regime.

This presentation will impact the forensic science community by providing relative frequencies of skeletal trauma in the Tuskulenai case, which can be used to locate People's Commissariat for Internal Affairs (NKVD) violence within a global framework of state-sponsored violence. This project also demonstrates that state agents may not adhere to state guidelines as closely as historical data suggests.

Following the Second World War, the NKVD represented the Soviet security apparatus responsible for arresting and punishing "enemies of the state." From 1944 until 1947, the NKVD executed 767 prisoners in the Lithuanian Soviet Socialist Republic (L.S.S.R.) and buried their remains in mass graves at the Tuskulenai Estate. Guidelines for prisoner treatment and execution were established by Soviet authorities to be implemented at a local level by state agents, including in the L.S.S.R. In particular, the penal code of the Soviet Union mandated that the only legitimate means of performing executions was by fusillade, or firearms, to the back of the head. This study examined violence committed by state agents, investigating their actual adherence to state guidelines. Specifically, this project analyzes the sex, age, and frequency of skeletal trauma in a sample of prisoners in order to understand how patterns of violence differ between two primary execution squads in the Tuskulenai case. Execution squads include those led by NKVD agent Vasilij Dolgirev (operating from November 1944 until October 1946) and Boris Prikazchikov (operating from November 1946 until April 1947). Based on historical data, no difference in trauma patterns was expected between state agents who implemented violence. This project also attempts to shed light on how state-sponsored violence toward Soviet citizens changed during the 1930s and 1940s. Thus, skeletal trauma in the Tuskulenai case is compared to other instances of Soviet violence, including those at Vinnytsia (Ukraine), Katyn (Russia), and Rainiai (Lithuania). Data is collected with the intent to compare patterns of violence in the Soviet Union with other instances of state-sponsored violence during the twentieth century.

Preliminary analyses in the Tuskulenai case reveal that approximately 95% of prisoners are male, and consist mostly of young adults (45%) or middle adults (38%). Frequencies of skeletal trauma differ between the execution squads. The majority of prisoners executed by Dolgirev's squad exhibit gunshot wounds (91%), followed by blunt force trauma (16%), and undetermined trauma (42%). In contrast, prisoners executed by Prikazchikov's squad demonstrate a lower frequency of gunshot wounds (57%), but a higher frequency of sharp force trauma (1%), blunt force trauma (31%), square defects (31%), and undetermined trauma (60%). When the relative frequencies of trauma are combined for the Tuskulenai samples, preliminary analyses demonstrate that 80% of individuals exhibit gunshot wounds ranging from one to four shots, while blunt force (21%), square defects (11%), sharp force (1%), and undetermined traumata (47%) are also observed. The frequency of gunshot wounds in the Tuskulenai case is lower than that of Vinnytsia (100%) and Katyn (100%), but higher than that of Rainiai (14%).

Thus, while historical data characterize these execution squads as operating uniformly in accordance with state guidelines, skeletal data indicates that executioners may differ in their compliance with state guidelines. Furthermore, comparison of the Tuskulenai case with other instances of atrocity demonstrates the range of variability in state-sponsored violence in the Soviet Union.

State Violence, Executioners, Skeletal Trauma

H56 Our Place in the Sun—Investigations Into the Boot Hill Cemetery at the Florida School for Boys

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After attending this presentation, attendees will understand how to use multiple methods of remote sensing, archaeological field methods, archival research, and forensic interviews to locate clandestine burials. Attendees will also learn about methods for reconstructing a historical narrative for humanitarian missions.

This presentation will impact the forensic science community and others by offering research into the lives and deaths of more than 80 children, which have previously not been reported. This research was undertaken as a humanitarian initiative for families searching for missing relatives. The results of the initial phase of this effort are reported here and have the potential to impact future investigations into the school.

In 2011, the Arthur G. Dozier School for Boys (a.k.a., "Florida School for Boys"), located in Marianna, Florida, was closed after more than a 100-year history of controversy regarding abuse, financial scandals, and allegations of murder. The Florida State Reform School first opened in 1900 and is located on 1,400 acres of land. Children were committed to the school for minor offenses such as "incorrigibility" or "truancy" or for serious crimes such as "theft and murder." Originally, the school housed children as young as six years old, including both males and females. Beginning in 1901, reports surfaced of children chained to walls in irons, being brutally whipped, and hired out for labor. During the first thirteen years of operation, there were more than six state-led investigations. Allegations of beatings, rapes, and whippings by more than 300 men, called the "White House Boys," led to a more recent investigation in 2008 – 2009 by the Florida Department of Law Enforcement (FDLE). The FDLE report cites newspaper and archival resources and lists 81 deaths that occurred.

Ledgers found in storage, newspaper reports, and death certificates researched through this project show 86 deaths resulted from 1911 – 1973. Among these, records indicate that 21 individuals were buried in the cemetery (1914 – 1952) and nine entries indicate that the bodies were "shipped" away. Therefore, the final depositions of more than 50 children are still unknown. In 1996, 31 metal crosses were placed within the current boundaries of the Boot Hill Cemetery; however, their locations do not correspond to actual interments.

The purpose of this research was to conduct a pedestrian survey, map the Boot Hill Cemetery, and to research the history of the site to determine the number, location, and identity of graves. Specifically, the initial goals of this project were to: (1) document and map the cemetery; (2) identify graves through multiple forensic and archaeological methods, including remote sensing using ground-penetrating radar; (3) research the site's history, creation, and use based on anthropological methods of field investigation; and, (4) research the identity of those buried in the cemetery and the morbidity and mortality patterns of the decedents.

Through the course of this investigation, the number and location of graves present within the cemetery were determined and the potential presence of multiple burial areas was investigated. Clandestine graves in the wooded areas surrounding the current Boot Hill Cemetery were found as far as 20m from the current site. Moreover, the identity and circumstances surrounding the deaths, based on archival research and forensic interviews, establishes patterns among those who died at the school such as a higher number of African American juveniles, a high number of runaways, and the spread of several infectious disease outbreaks. This presentation will detail the multiple investigative methods used and will provide qualitative results. Further, results from remote sensing followed by archaeological trenching and soil chemical analysis, which clearly demonstrate the presence of burial shafts, will be discussed. Finally, the historical significance of human and civil rights violations in Florida in the area of juvenile justice and the rights of

families to have accountability and transparency are discussed.

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Forensic Archaeology, Forensic Interview, Historic Cemetery

H57 The Use of an Alternate Light Source for Detecting Skeletal Material Under Water

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After attending this presentation, attendees will understand how a submersible Alternate Light Source (ALS) can be used to locate skeletal material underwater.

This presentation will impact the forensic science community by improving the search methods used by forensic divers and increasing the quantity and efficiency of evidence recovered.

When searching underwater crime scenes or disaster scenes for human remains, it may be advantageous for forensic divers to be able to detect the presence of skeletal material among other non-skeletal marine materials (such as shells and rocks). In terrestrial environments, this can typically be accomplished by visual and instrumental methods, but underwater conditions make it difficult to employ segregation techniques in these environments. This study investigates fluorescence of skeletal and non-skeletal materials using a submersible ALS and concludes that an ALS can be a useful tool for detecting skeletal remains in underwater searches.

Tests were carried out using a hand-held, integrated-battery light source advertised as submersible to 100 meters and saltwater resistant. Skeletal material examined included human bones, burned human bone, non-human bones (pig, deer, turtle, and fish), and non-human teeth (pig and dog). Other non-skeletal marine material examined included gastropod shells, bivalve shells, echinoderm shells, coral, rocks, and beach glass. The first stage of testing involved an investigation of whether skeletal material (including both bones and teeth) could be differentiated from non-skeletal marine materials using the selected ALS in a terrestrial environment. This phase was performed using ultraviolet and 450nm wavelength settings and filters in red, orange, and yellow. The second testing stage involved deployment of the ALS underwater to examine the ability to differentiate skeletal material underwater, and also to evaluate the practicality of using an ALS system (including light source and filter) underwater. The ALS was deployed in a freshwater lake where the water depth was approximately 15ft, and the device was tested at a depth of approximately 4ft. Test specimens were placed in a mesh bag, and observations were made using a diving mask fitted with a yellow filter.

In terrestrial tests, skeletal material could be easily differentiated from the other materials using the 450nm setting and a yellow filter on the basis of the intensity of fluorescence. Under these conditions, skeletal samples fluoresce yellow to orange, while all other materials failed to fluoresce, appearing dark purple/blue or simply dark. In underwater tests, the device functioned well, proving to be water resistant and effectively projecting light at a distance of up to several feet. Because the test was conducted in the daytime in shallow water, the light was found to be difficult to detect in bright areas near the surface. When the device was used in a dark crevice, however, the skeletal material could be seen to fluoresce. These results suggest that the technique is likely to be most effective at greater depths or during nighttime investigations.

While this technique can distinguish skeletal material from other materials, it does not distinguish human skeletal material from that of other animals that may be encountered in an underwater environment. In cases where bones or teeth are identified by divers using the ALS, the specimens will still need to be brought to the surface to determine their ultimate forensic significance. Nonetheless, the technique may be useful in eliminating the unnecessary collection and transportation of non-skeletal material to the surface. Although examined for its utility underwater, the ability to distinguish skeletal from non-skeletal material in a laboratory environment may also be useful in forensic anthropological segregating exercises.

Further studies aimed at how long skeletal material underwater retains its fluorescent properties would be useful, as would additional tests of the depth and visibility limitations (if any) of the device. It is concluded that the use of ALS under the conditions described may be very useful in forensic diving for detecting the presence of skeletal material in underwater environments.

Alternate Light Source (ALS), Forensic Diving, Forensic Anthropology

H58 The Use of Radiocarbon Analysis in a Chilean Human Rights Commingled Case

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After attending this presentation, attendees will understand potential values of radiocarbon analysis in identifying recovered commingled remains, particularly those that date to within the radiocarbon bomb curve, such as those of human rights interests in Chile.

This presentation will impact the forensic science community by showing how radiocarbon analysis was used to determine the relationship of commingled remains to individuals missing during Chile's military dictatorship.

The period of military dictatorship in Chile between 1973 and 1990 produced 3,227 qualified fatal victims, of which 1,465 were detained and missing; from these, 364 correspond to executed without repatriation of the remains. Detention centers were distributed throughout the country. One of them, located in the north, named "Campamento de Prisioneros Pisagua" (Pisagua Prisoners Camp), was in use from September of 1973 to October of 1974, and it is estimated that it held more than 800 prisoners.

In 1990, as a result of an ongoing investigation, a mass grave situated in the vicinity of the cemetery of Pisagua was exhumed. This mass grave contained the remains of 19 individuals, who were all in a state of natural mummification due to the arid and hot conditions of the local climate. Apart from the 19 individuals, who were promptly identified, a commingled group of human remains were recovered and, at the time of the exhumation, named "Bolsa 20" (sack 20). The archaeologist in charge concluded in his report that these remains were not related to the event of the mass grave and the remains were kept in custody by the Forensic Service of Chile.

Although "Bolsa 20" was reported as being inconsistent with any human rights case being investigated, family members solicited a re-evaluation of the case. Particularly involved with the case was the family of Michel Nash Sáez. He was a 19-year-old conscript who was arrested on September 11,1973, transported to the Campamento de Prisioneros de Pisagua, and executed on the 29th of the same month along with five other persons who were under arrest at the same center. Since three of the six bodies were found in the mass grave exhumed in 1990, the family hoped that Michel Nash might be represented in the remains contained in Bolsa 20.

A historical review of the case, including videos, medicolegal reports, and documentation was performed. Following standard anthropological analysis of the remains, it was decided to use radiocarbon analysis as a first approach and, depending on the results, genetic analysis might be attempted.

Radiocarbon analysis, especially in relation to the modern bomb curve, is particularly useful in the Chilean human rights cases since the events of human rights interest date after 1973. The elevated levels of

^{14}C in the atmosphere produced by atmospheric testing of thermonuclear devices reached a peak in 1963 and then began a gradual decline due to the termination of such testing by the United States, Great Britain, and the former Soviet Union.

A minimal number of individuals of three was established, two of them adults and one subadult. The value of "percent Modern Carbon" (pMC) for individual 1, from soft tissues, was 97.7 ± 0.4; for individual 2, from soft tissues, 73.5 ± 0.3; for individual 2, from bone, 73.3 ± 0.3; and for individual 3, from bone, 64.4 ± 0.3. All of these values fall below those of the modern bomb curve and are inconsistent with those predicted if the individuals involved died in 1973.

The contents of "Bolsa 20" were excluded as belonging to any victim from the period 1973 to 1990. In fact, the remains could be dated using conventional radiocarbon dating as follows: individual 1 – 1840 to 1900 A.D.; individual 2 – 550 to 490 B.C. (from the soft tissue sample), 570 to 510 B.C. (from bone); individual 3 – 1900 to 1980 B.C.

This case constituted the first to use radiocarbon analysis in a human rights case in Chile. Subsequently, this methodology has been used in more than 50 cases.

Radiocarbon Analysis, Missing, Chile

H59 Computerized Reconstruction of Fragmentary Skeletal Remains

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After attending this presentation, attendees will gain an understanding of the process of fragmentary remains reconstruction using computerized methods and the use of Computed Tomography (CT) and 3D models to sort commingled fragmentary remains.

This presentation will impact the forensic science community by describing the development of a software program to facilitate the determination of the Minimum Number of Individuals (MNI) and make metric assessments of sex, ancestry, and stature from statistically sound bone reconstructions of fragmentary remains. Error rates in the form of a confidence score based on root mean square error will be established for the bone reconstructions, as well as for the computer-automated measurements generated by the software.

Within the medicolegal system, forensic anthropologists perform the essential task of creating a biological profile to aid law enforcement in identifying unknown human remains. The four primary components of the biological profile are age, sex, ancestry, and stature. The parameters of the biological profile are intricately interwoven in that, frequently, one component is necessary in order to make precise determinations about other components. In cases of mass disasters or commingled assemblages, the determination of individual biological profile parameters is complicated by the presence of multiple unassociated elements. The ability to make biological profile assessments using isolated bones or bone fragments is critical. Although developed independently, the 3D approach to the quantification of commingled remains is a logical extension of coding and two-dimensional methods developed in zooarchaeology and bioarchaeology.[1-3] Recent work quantified small fragmented remains into an Osteological Information System (OIS) using Geographic Information System (GIS) software to derive Minimum Number of Elements (MNE) values and MNI estimates.[3] These systems are time consuming and depend on the observer to manually digitize each fragment into the OIS application. The resulting image provides an MNE estimate for the element under investigation. The proposed application provides a system to perform such analyses and to manage complex mass disaster cases or commingled bone assemblages.

In order to enable the computerized reconstruction of fragmentary remains, a new method was developed to match fragmentary remains with 3D template bones for the pelvis, humerus, femur, and cranium. These template bones are average bones generated from a training set with homologous points on the 3D surfaces. In order to generate such homologous points, the 3D models had to be added to a statistical atlas[4] that redistributes the points on the bone surface to ensure correspondence among landmarks.[4] Fragmentary remains are then matched to each template bone using surface descriptors. Outputs of this process are fragmentary pieces that are registered together in space. The next step involves reconstruction of a full bone by interpolating missing data between registered pieces. This step is enabled by optimizing the principal components calculated from the training set. In order to develop and test the system, a highly fragmentary commingled sample was used as a proxy for a mass disaster: the Morton Shell Mound osteological collection. The Morton sample represents over 25,000 human bone fragments from approximately 125 individuals. At the time of submission, ninety catalog numbers containing 15,133 human bone fragments were sorted by element, of which 1,054 fragments have been CT scanned and isolated for analysis.

Upon completion of this study and finalization of the software, all scanned skeletal remains from each scene will be reviewable within the application. An estimate of MNE of the scanned material will be provided following osteological protocols developed in forensic anthropology and bioarchaeology.[3] MNE estimates will allow for the determination of MNI. Finally, the application calculates a combination of traditional anthropological measurements and frequently used biomedical measurements. The traditional measurements can be used in a program such as FORDISC 3.0 or manually entered into equations used for the metric assessment of the biological profile.

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Fragmentary Remains, Commingled Remains, Computer Modeling

H60 Spatial Analysis and Modeling of Missing Persons Burial Locations in Multiple Armed Conflict Contexts

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After attending this presentation attendees will be familiar with a new application of geographic analysis to search for missing persons who have been killed during armed conflict and are presumed to be buried in unmarked graves. Attendees will gain an understanding of the geographic trends of burial locations—despite varying contextual circumstances—that can assist the search for missing persons, which is often directed by forensic anthropologists. Attendees will also gain an appreciation for geographic information science tools, which have

seldom been exploited by forensic anthropologists. Such techniques are of increasing importance as the field of forensic anthropology becomes more standardized, particularly with respect to the use of archaeological methods.

This presentation will impact the forensic community by introducing innovative techniques available to those who are tasked with locating missing persons in armed conflict contexts. A thorough understanding of how to use geographic information science approaches and software packages for forensic research can provide interesting insights into the spatial behavior of killers, reduce field costs for contemporary operations, and help locate older burial sites in locations where witnesses are not forthcoming or several years have passed since burial. The location of remains is the first critical step in identifying remains for the purposes of death investigation and repatriation.

Only in recent years have forensic anthropologists adopted geographic methods to address problems often confronted in forensic anthropological research and casework, such as burial prospection or spatial distribution of surface remains.[1-3] This study builds upon and expands previous research on clandestine burial location analysis from the Spanish Civil War.[4] The study's hypothesis posits that limited resources in times of armed conflict and rational human behavior result in cross-context patterns of victim burial location. The material being presented is a subsample of a study of burials in seven countries/conflicts. This presentation includes analysis of burial site characteristics of three conflicts (Spanish Civil War 1936 – 1939; Bosnia-Herzegovina 1992 – 1995; and Korean War 1950 – 1953) and examines burial locations of both combatants and non-combatants. The study sample includes 155 burial site locations and uses geographic information software and methods including kriging, viewshed analysis, and Ripley's k-function cluster analysis. These methods are used to ascertain the geographic characteristics of burial locations (e.g., land use, distances from roads and populated areas, visibility of burial location, distances traveled between site of disappearance/death/burial), demonstrating the types of locations chosen for burial. In this study, preliminary results show strong inter- and intra-context consistency given certain contextual variables. This study also indicates that there is a general evolution of burial site selection from less clandestine (e.g., cemetery burials) to more clandestine (e.g., very rural or mountainous terrain) locations that is dependent upon prevailing socio-political factors, and the course of a particular armed conflict. Changing burial activities include the postmortem movement of bodies from primary to secondary locations. Secondary burial locations, however, are highly dependent upon motives for body relocation. Being able to ascertain such motives informs greatly upon geographic location characteristics that should be sought when searching for missing persons' remains.

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Burial Prospection, Missing Persons, Spatial Analysis

H61 Sampling Procedure for the Histological Analysis of Pediatric Skeletal Trauma

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After attending this presentation, attendees will appreciate the use of hard tissue histology in evaluating pediatric trauma and be introduced to a skeletal sampling procedure that forensic anthropologists should consider when using bone histology to examine pediatric remains.

This presentation will impact the forensic science community by demonstrating the growing role of anthropology in the recognition and interpretation of pediatric fractures and the importance of skeletal sampling procedures to maximize evidence obtained during the skeletal survey.

The evaluation of fractures in children is vital for interpreting an injury as non-accidental and in determining if a history of injury exists. Thus, complete documentation of pediatric skeletal injury is paramount in diagnosing abuse.[1] The common anthropological approach to skeletal analysis is to remove the soft tissue and other organic components using a maceration procedure, leaving dry bone for analysis. Love and Sanchez discussed an approach to grossly examine the pediatric skeleton that requires the meticulous reflection of soft tissues at autopsy to expose the osseous and chondral tissues.[1] In addition to this approach, sampling fracture sites or potential fracture sites for histological analysis may identify fractures not seen during the gross examination or provide useful information as to the mechanism and/or timing of the injury that would be otherwise lost due to the maceration procedure. Furthermore, the typical anthropological assessment of fracture healing relies upon the observation of gross bony changes, which will appear secondary to histological changes.

Owing to the difficulty in evaluating pediatric skeletal trauma, a sampling technique was developed to remove specific fractures or segments of fractures for histological analysis as a part of the anthropological exam. The first step is performing a radiological and gross skeletal survey to determine if histological analysis would be beneficial and, if so, select the sampling area(s). The following criteria assist with the sample selection: Sampling is considered for (1) fractures with no gross evidence of healing; (2) fractures that demonstrate differential healing; (3) areas overlying hemorrhage with no apparent bone injury; and, (4) bones suspected of injury that contain chondro-osseous junctions.

Following the gross evaluation and documentation of a selected sampling site, the sample is removed for histological preparation. A small rotary tool with a cut-off wheel is used to remove a segment of the fractured bone. For example, a segment of a skull fracture is removed by cutting a window out of the fracture. An alternate method is to remove the bone in its entirety. Following specimen removal, the tissue is fixed in a 10% formalin solution for 24 hours and submitted to the Histology Laboratory within the Medical Examiner's Office for decalcification. Depending on the specimen, decalcification may take 1 – 3 days.

Once decalcified, the sample can be trimmed using a microtome blade or scalpel in order to fit the sample into a histological cassette for embedding. It is important to leave a portion of "normal" or non-traumatic bone on either side of the fracture so one can examine the lamellar bone leading to the fracture margins or callus, if present. The sample is then placed into the histological cassette and positioned for the desired histological section (e.g., cross section or longitudinal section). The samples are embedded into paraffin blocks and slides are prepared following standard histological procedures. A hematoxylin-eosin stain is applied to assist with the identification of histological features (i.e., new bone formation, mineralized callus, cartilaginous tissue, or fibrotic tissue). Finally, the anthropologist reviews the histological slides with the pathologist to evaluate any microscopic findings.

While this technique requires additional time to perform the analysis of pediatric skeletal trauma, it provides further opportunity to describe fractures that may be difficult to identify or fully characterize at the gross and radiological level. In addition, histological analysis may provide for a more accurate determination of the timing of an injury considering that

early reparative response to a bone fracture will not manifest at the gross level until osteoblasts and osteoclasts are given time to respond to the injured tissue. Therefore, the sampling technique described should be considered by anthropologists working with pediatric remains to ensure that skeletal injuries are fully characterized.

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Pediatric Trauma, Histology, Fracture Analysis

H62 Histopathology of Ante- and Peri-Mortem Infant Rib Fractures

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WITHDRAWN

H63 Use of Silver Nitrate Stain to Visualize Microanatomical Features in Decalcified Bone Cross-Sections

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After attending the presentation, the attendees will learn about slide preparation modifications that can be used for visual microanatomical structures on decalcified bone cross-sections.

This presentation will impact the forensic science community by providing a technique for forensic anthropologists to continue their analyses using bone histology without the need for specialized equipment. Additionally, these slides can be prepared within the histology laboratories employed by medicolegal offices.

The use of histological techniques to assess bone is well established in anthropology. It is especially valuable in the applied field of forensic anthropology for the differentiation of human from non-human bone, age estimation, differential diagnosis of bone pathological conditions, and assessment of overall bone health. The standard petrographic technique for producing thin sections typically used by anthropologists involves undecalcified bone samples that are vacuum embedded in molds filled with epoxy resin and hardener. Once hardened, 1mm sections are cut from the embedded sample block using a diamond-blade saw and ground to a thickness of approximately 70µm. Due to the expense of specialized equipment and supplies, the petrographic technique is not generally available at medicolegal offices. The standard bone histological technique used in medicolegal offices to produce thin sections involves cutting decalcified bone into sections approximately 4µm thick using a microtome and staining with hematoxylin and eosin. Unlike petrographic sections, standard histological sections of decalcified bone are too thin for visualization of cement lines, a key feature used by forensic anthropologists to identify osteons and fragments. Previous studies have shown that silver nitrate stains can successfully be used to demarcate cement lines. Since silver nitrate adheres to calcium and darkly stains calcified tissue, the contrasting unmineralized cement lines remain unstained. This study explores the use of silver nitrate-stained decalcified sections in histomorphometric analyses of bone for the purposes of age estimation.

Samples from the anterior third of the sixth rib were collected from unidentified decedents who were exhumed as part of the Harris County Institute of Forensic Sciences Unidentified Decedent Review Project. Sections were prepared using the standard histological procedure where the samples were formalin-fixed and decalcified prior to embedding in paraffin wax. Once set, 8µm slices were sectioned from the paraffin block and placed on a slide. Eight micron sections were selected because they are thick enough to provide sufficient contrast between bone tissue and cement lines without causing the section to lift off the slide, as can happen with thicker decalcified bone sections. The sections were treated using a silver nitrate stain and cover-slipped. Once the slides were prepared, age was estimated for each sample following the Stout and Paine method as well as Cho et al. method using anterior cross-sections of the sixth rib.[1,2] These estimates were compared to the age estimated for the decedent using macroscopic techniques, in particular the sternal rib method and the pubic symphysis method.[3,4]

Histological age estimates derived from the decalcified sections, particularly using the Stout and Paine method, were within or close to the macroscopic age estimates for the decedents in the sample population.[1] The histological point estimate of age obtained for each rib was compared to the phase mean assessed for the pubic symphysis and/or sternal rib methods. Although there were differences in estimates between the microscopic and macroscopic groups, no consistent pattern was observed. The histological point estimate of age either fell within the 95% confidence interval for the macroscopic methods or exceeded the interval, therefore producing an overestimate. Since there is no clear understanding of how histological age estimates derived from petrographic slides compare to the macroscopic techniques, the efficacy of the decalcified method compared to the petrographic method could not be evaluated. However, because the age estimates are relatively close, these findings suggest that the decalcified silver-stained sections are appropriate for use in histological age estimation and the method should be explored further.

A limitation of the method is the use of extremely thin bone sections that have a tendency to fold when mounted on a slide, thus reducing the amount of bone that can be read. Tears and obliteration of the periosteal surface was also noted in some slides.

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Bone Histology, Decalcified Bone, Stain

H64 Reconstructing Taphonomical History in Osseous Remains From the Korean War Using Histology

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After attending this presentation, attendees will have knowledge of how different taphonomical processes affect the preservation of bone. Additionally, the aim of this presentation is to show how these processes can be identified using bone histology and how this can contribute to reconstruct taphonomic history of bone.

This presentation will impact the forensic science community by contributing to knowledge on taphonomic processes affecting bone microstructure preservation and how this can help reconstruct taphonomic histories of bone.

Taphonomic processes can alter or destroy information available from skeletal remains. Much research has been done to evaluate bone preservation, to identify diagenetic pathways as well as develop methods to screen for sample quality. One of the research methods that can be used to identify type and extent of bone degradation is thin

section histology. The different processes that influence bone preservation leave behind histological features that are preserved through time. This makes it possible to reconstruct taphonomical histories.[1,2] Factors that can be observed using histology are type and extent of microbial alteration, physical damage (cracking), and inclusion or infiltration of exogenous material. The latter can be very informative of the burial environment of the bone; for example, certain minerals only form under specific environmental conditions. Microbial alteration usually occurs relatively soon postmortem and is less dependent on environmental factors but seems to be influenced by deposition/burial methods. Certain types of microbial alteration are indicative for deposition in different (aquatic) environments or can indicate presence of oxygen in the environment (fungi). The presence or absence of bacterial degradation of bone has been linked in archaeological studies to differences in early postmortem treatment of remains.

As taphonomic signatures are preserved through time, information can be gained from their presence or absence. In this study, the taphonomy of remains from the Korean War, which come to the Joint POW/MIA Accounting Command-Central Identification Laboratory (JPAC-CIL) generally from three very different contexts, is characterized. Some of this material was recovered in the Democratic People's Republic of Korea (DPRK). Other samples come from a commingled assemblage of remains that were turned over by the DPRK to the United States government. A third category of samples comes from Korean War remains that have been buried shortly after the war as "unknowns" at the National Memorial Cemetery of the Pacific. Bone samples were examined from these three very different preservation scenarios using histology to identify diagenetic features. The main goal of the study is to characterize the effects of the different postmortem scenarios on bone preservation, and to elucidate the taphonomic history for these samples. Specifically, the effect of secondary burial on bone preservation will be discussed, and how this could be identified through patterns of taphonomic features.

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Histology, Taphonomy, Bone

H65 Validation Dental Cementum Increment Analysis for Determining Season at Death

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After attending this presentation, attendees will understand the method of determining season at death using dental cementum increment analysis. Attendees will be able to explain how use of the method helped identify a cold case homicide victim.

This presentation will impact the forensic science community by providing criminalists, anthropologists, and odontologists with one more method to use in gathering evidence from unidentified human remains.

Dental cementum research was once exclusively the domain of zooarchaeologists who used the biology of cementum to determine the ages and seasons at death of mammals at archaeological sites.[1] Dental cementum forms the outermost layer of tooth roots and it binds to the periodontal ligament to hold teeth in their sockets. Cementum is not remodeled like bone, rather it is deposited and mineralized throughout adulthood. In humans, cementum is laid down in pairs of bands: one light or translucent band is laid down in the spring/summer and one dark band is laid down in the winter/fall. The layers of accumulated pairs of bands of cementum are used to determine age at death by adding the number of pairs of bands to the age at which the tooth erupts.

The applications and limitations for estimating age at death in humans using this method has been established previously; however, the use of dental cementum to estimate season of death was only recently evaluated.[2-6] Therefore, a pilot study was devised to test the applicability of using the color of the outermost band of cementum to determine season at death in humans by using 112 teeth donated by the patients of an oral surgeon.

Extracted teeth were embedded, sectioned, ground and polished, and examined under transmitted polarized light microscopy to great results: the color of the outermost cementum increment correlated with the season at death 99% of the time. Teeth extracted between October and April exhibited a dark outer band, thus, a fall/winter season. Teeth extracted between April and October exhibited a light outer band, therefore, a spring/summer season.

To confirm the results of the pilot study, 450 teeth were chosen at random from 1,300 teeth donated by patients of the Creighton University School of Dentistry. In addition to increasing the sample number, the geographic location from where the donated teeth originated differed, too. Creighton University is located in Nebraska, and Omaha has a very different climate than coastal Santa Cruz, California. Of particular curiosity was whether local weather differences would matter. Would the Nebraska teeth sort into the same broad seasons as had been identified in the Santa Cruz pilot study?

Analyses were performed in the blind, and it was not until the teeth had been embedded, sectioned, ground and polished, and data collection completed that the exact dates of extraction were made known to the investigators. Date of extraction was used as a proxy for date of death. Results of the study indicated that climate bore no effect on estimating season at death from dental cementum. The Nebraska sample sorted into the same two broad seasons as the Santa Cruz sample in 440 of the teeth, while 10 of the teeth yielded indeterminate results. Depicted in this paper are tables demonstrating the seasonal bands increasing in thickness from the beginning to the end of the season. Scatter plots form the basis of a discussion as to whether four seasons at death rather than just two are indicated.

Dental cementum increment analysis has now been used to determine season at death in a dozen forensic test cases in California. In one particular case, both age and season at death were determined for a 37-year-old cold case homicide. The remains of a young woman had been recovered in 1971 bearing evidence of sharp force trauma. Her remains went unidentified until 2008 when the remains were exhumed and reanalyzed for identification. Anthropological assessment indicated an age at death of 23 – 30 years based on medial clavicle and iliac crest epiphyseal fusion. Dental cementum increment analysis indicated an age at death of 23 – 25 years and a spring/summer season at death. The revised profile, along with a forensic facial reconstruction by Gloria Nusse, was posted to the Doe Network's web page, and a maternal relative came forward. The victim was 24 when she had gone missing in late August/early September.

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Dental Cementum, Season at Death, Validation Study

H66 Does Cut Direction Affect Increment Viewing in Dental Cementum Increment Analysis for Age and Season at Death?

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The goal of this presentation is to compare two methodological preparations of dental cementum histological slides of the same specimen side by side to test whether cut direction affects cementum increment in determining season at death.

This presentation will impact the forensic science community by demonstrating how the validity of dental cementum incremental analysis on human teeth needs to be evaluated and expanded to answer which technique is most useful in determining season at death in humans. Of the same specimen, a transverse cut is found to yield readable seasonal information than its longitudinally cut counterpart.

Cementum is the tissue that binds the tooth to the periodontal ligament and is deposited throughout the course of life. Because cementum is variably mineralized, its fundamental structure of collagen fibrils yields a banded appearance to cross-sections of the outer edge of the tooth, analogous to tree rings. In mammals, one pair of cementum bands is laid down annually.[1-3] In the spring/summer, mammals lay down a translucent or clear band while in the fall/winter, they deposit an opaque or dark band. Counting the number of pairs of bands and adding that number to the age at which the tooth erupts yields an age at death estimate that is more accurate than age estimates achieved by most skeletal methods (e.g., epiphyseal union, sacroiliac joint surface appearance, rib end morphology, etc.).[4,5] Pilot work by Wedel demonstrated by observing the outermost increment, season at death could be determined with 99% accuracy.[6] She further demonstrated that the seasonal transition of the outermost cementum band in humans from translucent to opaque occurs in October and then transitions back to translucent in spring/summer, beginning in April.[6] All adult teeth that are erupted and in occlusion do exhibit increments.[6] Hsieh and colleagues demonstrated teeth of any type (cuspids, bicuspids, and molars) can be used in determining age or season at death without significant differences in the results.[7]

Hsieh et al.[7] focused on distinguishing the outermost increment in transverse thin sections of the middle third of the root by using transmitted polarized light microscopy; however, Stein reported a high accuracy (r=0.93) in age estimation using longitudinal cuts instead of transverse cuts.[7,8] Other recent researchers used longitudinal sections, while acknowledging a discrepancy in the sectioning method among cementum researchers.[5,9,10] Some authors prefer the section to be longitudinal, whereas a majority of authors prefer transverse cross sections. Unfortunately, no author explicitly explains his or her preference for transverse versus longitudinal sectioning. Further, none of the researchers compare these two techniques side by side, sectioning one tooth both transversely and longitudinally to see if the results differ.

Therefore, it becomes necessary to test the accuracy of determining season at death based on the color of the outermost cementum band using both transverse and longitudinal cuts from the same specimen in a fairly large sample of teeth of known season at death (or season at extraction, which is the proxy for season at death). It is hypothesized that transverse cuts yield more accurate and reliable results than longitudinal sections. This hypothesis will be rejected in either of two circumstances: (1) there is no statistical difference between the longitudinal versus transverse section results; or, (2) if either the season at death from each tooth does not match the season from when it was actually collected from the patient. In other words, the research will only be considered helpful if a fall/winter band is recorded from a tooth collected in the fall/winter.

Fifty-five transversely-sectioned single-rooted teeth were randomly selected and then sectioned longitudinally. Season at death was accurately determined in all of the transversely sectioned teeth, whereas season at death was only correctly determined in 56% of the longitudinally sectioned cases. Further, the sample of 55 longitudinal cuts contained six indeterminate samples (~10% of cases) in which the outermost increment could not be observed at all.

Of these 55 transversely and longitudinally cut teeth, the transverse cuts yielded a higher number of correctly classified samples and fewer indeterminate samples than the longitudinal sections. The longitudinal cuts yielded a significantly higher number of indeterminate samples and, thus, fewer samples were correctly identified as to season at death. Qualitatively, the transverse cuts were easier to read than the longitudinal cuts. In other words, a transverse cut is more likely to yield readable seasonal information than a longitudinal cut from the same specimen. Therefore, a transverse cut is preferred for its better preservation of information and a better chance to result in a valid sample for the use of anthropologists.

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Season at Death, Cementum Increments, Transverse Cut

H67 Using Tooth Cementum Annulation for Age Estimation in Forensic Casework

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After attending this presentation, attendees will understand the basic premises behind age estimation using Tooth Cementum Annulations (TCA) and the current theoretical and methodological issues relevant to its application in forensic practice. The role of TCA, as an age estimator, in the analysis of two forensic skeletal cases will be discussed, including the methods used, documentation procedures, and analytical results.

This presentation will impact the forensic community by outlining the practical use of TCA in casework and make recommendations as to when it is appropriate to use as an age estimator. Further discussion as to how the results of TCA analysis should be conveyed to law enforcement is also suggested, using the case studies as examples.

Cementum is a dental hard tissue that anchors the teeth into their sockets via the periodontal ligament. Once a tooth has erupted, two bands of cementum are laid down per year of life, although the

phenomenon has been observed in unerupted teeth as well. Under magnification with transmitted light, the bands alternate light and dark in color, with each pair of bands representing one year.

The phenomenon of cementum increments is well documented in some non-human populations and does appear to show sensitivity to life history variables like birth, trauma, and nutrition. Although why cementum increments are initiated into production and their apparent circadian rhythm remains somewhat speculative, especially in humans, cementum annulation has been proposed as a forensic aging method. There are many fundamental questions that are as yet unanswered or theoretical in nature, but cementum annulation is observable and does correlate with age at least as well as other traditional aging methods, making it attractive as another tool in the forensic anthropologist's toolkit.

Case 1 represents a completely skeletonized cranium, mandible, atlas, axis, and hyoid (body only) discovered in a partially decomposed duffel bag with a closed zipper. Based on dental eruption, cranial sutures, and general condition of the limited bones recovered, age was estimated to be "adult," approximately 25 to 50 years old at the time of death. Case 2 represents a partially skeletonized female with extensive blunt force trauma to the cranium. The skeleton, originally discovered and analyzed in the early 1990s, was estimated to represent a female of European ancestry, aged 18 to 24 years. During re-evaluation of the case in 2009 for additional leads and the potential for new forensic techniques, it was discovered that the postcranial skeleton, including the mandible, had been cremated; however, the cranium was still in evidence. In each case, a tooth was analyzed for TCA.

The contribution of TCA to each of the cases was very different. In Case 1, TCA as a 'traditional' age indicator, was employed. Given the lack of other specific age indicators, TCA contributed to a narrowing of the original age estimate, from 25 to 50 years to 30 to 40 years old at the time of death.

Case 2 involved a much different role for TCA. Only 2.5 pairs of bands were observed on the tooth. When added to the average age of eruption for the tooth, a much younger age at death than was originally reported was indicated. Taking a holistic approach and integrating all age indicators available, a re-evaluation of the first skeletal analysis was prompted. Although the postcranial skeleton was cremated, excellent documentation and photographs were available so that the raw data on which the first biological profile was based was sufficient to use. During the second analysis, more inclusive, updated standards were employed, as well as a critical approach to the methods used in the first analysis. Ultimately, TCA contributed mostly to prompt an updated look at the case and appraisal of the skeletal indicators, which suggested an age between 14 and 18 years at death.

These cases illustrate the potential use of TCA; however, a realistic appraisal of TCA and the assumptions on which is it is based, both proven and unproven, need to be explicitly addressed before TCA is widely used in a forensic setting and to direct future research.

Cementum Annulation, Anthropology, Age-at-Death

H68 Femoral Midshaft Histomorphometric Patterning: Improving Microscopic Age-at-Death Estimates From Adult Human Skeletal Remains

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After attending this presentation, attendees will understand how the use of microscopic techniques to estimate adult age at death is well established within physical anthropology's subfields of bioarchaeology and forensic anthropology, but diligent work is needed to overcome the long-standing problems of the Osteon Population Density (OPD) asymptote and relatively high Standard Error of the Estimate (SEE).

This presentation will impact the forensic science community by suggesting how the posterior Region Of Interest (ROI) can be utilized for production of the most accurate microscopic age-at-death estimates from adult human skeletal remains, and thus, for better understanding of the adaptive success of past populations by bioarchaeologists, and for more positive identifications by forensic anthropologists.

Review of the microscopic age-at-death estimation literature reveals that arbitrarily changing skeletal elements, histological variables, sample demographics, and sampling locations have not allowed for accurate age estimation of individuals over approximately 50 years or reduced the standard error of age estimates. This investigation, therefore, began with substantiated theory. All healthy, mobile femurs have in common: genetic programming to establish initial size and shape; the developmental processes of endochondral ossification, appositional growth, and modeling; biomechanical adaptation; periosteal adaptation; cortical thinning and shape change during aging; mechanosensation and mechanotransduction; and bone remodeling.

Building from this theoretical knowledge base, it was first hypothesized that topographical variation in remodeling exists around human femoral midshaft periosteal cortices that reflects the constraints of normal anatomical development, customary biomechanical usage, and standard mechanobiological functioning. Second, it was hypothesized ROIs associated with the I_{min} second moment of area biomechanical axis would exhibit the lowest remodeling as a result of minimal biomechanical loading. Third, it was hypothesized remodeling at biomechanical ROIs would be histomorphometrically more consistent than at anatomical ROIs due to unchanging functionality. These hypotheses were tested by counting remodeling events at eight standardized periosteal ROIs (four anatomical—A (anterior), P (posterior), M (medial), L (lateral) and four biomechanical—I_{maxAnt}, $I_{maxPost}$, I_{minMed}, and I_{minLat}) of 200 adult femoral midshaft cross-sections originally harvested by M.F. Ericksen from George Washington University dissecting room cadavers. The sample was specifically composed of 98 males and 102 females, largely of European descent, ranging in age from 30 to 97 years.

While no evidence was found for reduced remodeling at I_{min} ROIs or for more consistent remodeling at biomechanical ROIs, 14 statistically significant differences were found between ROI OPD medians indicating topographical variation in remodeling exists around the femoral midshaft. Specifically, the lowest OPD values were found to occur at the Anterior ROI, followed by the Posterior, I_{minMed}, $I_{maxPost}$, I_{maxAnt}, I_{minLat}, Medial, and Lateral ROIs. Additionally, although the anterior femoral cortex has traditionally been sampled for microscopic age at death estimation, here, the Anterior ROI was found to reach the OPD asymptote first and was associated with the highest SEE (± 11.542 years). Alternatively, the Posterior ROI was found to be associated with the lowest SEE (± 3.260 years) and second lowest median OPD value, but showed no signs of having reached the OPD asymptote. It therefore bears further investigation to see if this pattern can be replicated across multiple samples. If so, it is suggested the Posterior ROI be utilized for production of the most accurate microscopic age-at-death estimates from adult human skeletal remains, and thus, for better understanding of the adaptive success of past populations by bioarchaeologists, and for more identifications by forensic anthropologists.

Histomorphometrics, Age-at-Death, Regions of Interest

H69 Statistical Considerations of the Histomorphometric Test Protocol for Determination of Human Origin of Skeletal Remains

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After attending this presentation, the attendees will have a better understanding of a test protocol using osteon area as the basis for recognizing remains as likely human in origin. They will also receive an exposition of the philosophy underlying the statistics used in the test, and how that philosophy underpins interpretation of test results.

This presentation will impact the forensic community by explaining a new test protocol that is widely applicable and by clarifying the appropriate use of statistics in a forensic test.

Tersigni-Tarrant et al. proposed the use of osteon area as an effective measure for recognizing human bone versus other species.[1,2] It is widely known that humans have exceptionally large osteons on average, and that most species that share similar histological structure have smaller osteons. However, it is also clear that there is variation in individual osteon size within species, within individuals, and even within the same histological section. Any test that will utilize osteon size must have a proper statistical foundation. A test protocol has been developed that provides a significance test as the basis for interpretation of the osteon size. The test protocol requires one to: (1) section the case specimen and prepare a digital image of the section with scale; (2) import the image into the program Image J; (3) randomly select 30 osteons to measure; (4) measure the osteon areas and calculate the arithmetic mean; (5) test the null hypothesis that the remains are human by formal comparison (t-test) of the case specimen mean with the mean and standard error (with N=30) from a reference database consisting of 1,204 osteons from multiple long bones of five known individuals; and, (6) assess the strength of the evidence using Deborah Mayo's concept of severity.[3] A critical component of this protocol is the use of the randomly selected osteons as the basis for the test.

Human reference data has been analyzed and statistically compared to a close living relative, the chimpanzee (*Pan troglodytes*). This comparison is arguably a rigorous way to evaluate the power of the test to exclude non-human bone specimens. This test has also been applied to 37 specimens from 35 different known human individuals. Collectively, these efforts serve as validation of the test protocol. From the reference data (N=1,204 osteons), the mean osteon area was calculated at 37,298.5μm^2, standard deviation at 14.623.7, and noted that the distribution was skewed to the left (non-normal). The chimpanzee data (N=794 osteons from 17 individuals) showed a mean osteon area at 25,324.8μm^2, a standard deviation of 14,346.5, and a distribution skewed to the left. Bootstrapping was used to derive estimates of the means and standard deviations. The original reference data for each species was sampled with replacement 1,000 times, with N=30 each iteration. The mean was calculated for each iteration, which facilitated estimation of the population mean and offered a standard deviation of the estimated means. These values were: human mean at 37,365.3, standard deviation (of means) at 2,727.7; chimpanzee mean at 25,377.6, standard deviation (of means) at 2,692.1. Both sets of means exhibit a normal distribution. The closeness of the bootstrap means to the original estimated means was noted, as was the closeness of the bootstrap standard deviations of the means to the standard errors of the original estimates when basing it on a sample size N=30 (human standard error at 2,669.9; chimpanzee standard error at 2,619.3). Subsequent analyses employ bootstrap estimates since they are slightly more conservative than the original estimated means. The power of the test with the human mean as the null hypothesis and the chimpanzee mean as the alternate is approximately 0.90. The high power suggests that the test will have utility. Severity estimates were utilized to evaluate individual test results. Severity of a test, as defined by Mayo, is based on the probability that the test would reject the null hypothesis if in fact an alternative is true.[3] The more severe the test, the more likely one will reject the null when it is false. High severity justifies greater confidence in the null hypothesis when it is accepted, supporting the interpretation as "human" in many instances.

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Hypothesis Test, Statistical Power, Osteon Area

H70 The Use of Bone Histomorphology at the Central Identification Laboratory to Remove Nonhuman Remains From CIL Accessions

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After attending this presentation, attendees will understand how histomorphology is used to identify non-human osseous fragments at the Central Identification Laboratory (CIL) at the Joint POW/ MIA Accounting Command (JPAC) and how the techniques used at the CIL could easily be employed into a variety of analytical laboratories that deal with fragmentary osseous material.

This presentation will impact the forensic science community by providing a methodology for the accurate and rapid identification of non-human osseous fragments within any size laboratory.

The CIL has consistently focused on the use of various types of validated methods for the assessment of osseous remains. This includes the use of techniques such as metric analysis, chest radiographic comparison, and various types of DNA testing for the purposes of aiding in identification. Since 2006, the CIL has also incorporated histological analysis for the assessment of possible osseous remains as part of its analytic toolkit.

The CIL has nearly 1,000 accessions that contain biological material housed in its laboratories at any particular time. While turnover is high, the speed at which fragmentary cases are resolved is a bit slower than that of the intact remains. Some of these accessions contain highly fragmentary remains that have been excavated from crash sites. There are many cases, however, that are small fragments of bone with little to no provenience and little to no other loss association information. Histological analysis has helped immensely to cull the non-human/non-osseous materials in these highly fragmentary or information-scarce samples.

The utilization of histological and histomorphometric techniques has significantly reduced the number of non-human osseous remains that are part of accessions at the JPAC-CIL. Since the inception of the Standard Operating Procedure (SOP) for histological analysis, the CIL has been able to efficiently remove non-human remains from an enormous forensic caseload by assessing the histomorphometric features of the often fragmentary remains.

For example, between 2007 and 2012, 112 of 269 samples tested (approximately 42%) were able to be removed from the CIL's active accession lists as they had the characteristic hallmarks of non-human bone. The removal of this large proportion of remains from active accessions using a relatively rapid type of assessment has made a significant impact on the overall number of cases that are able to be resolved as definitively non-human or non-osseous, as well as remove material that has no forensic value for the identification of the individuals the CIL is charged with identifying.

The methodology for standard histologic analysis at the CIL utilizes three steps: embedding, sectioning, and analysis. First, basic epoxy embedding techniques are used to stabilize the fragment for thin sectioning. Next, using a thin-sectioning saw capable of making bone sections approximately 0.8mm thick, the specimen is cut transverse to its longitudinal axis. Finally, the thin section is attached to a glass slide, viewed under a standard light microscope at 50x magnification, and compared to our SOP decision matrix.

The current histomorphology SOP provides analysts with an avenue to efficiently rule out remains as human with a simple histological

analysis. The analyst can nominate fragments of bone for histological analysis in much the same manner as they would nominate specimen for DNA analysis. The remains are then assessed by an analyst competency-certified in histological techniques. The current SOP allows for the following analytical conclusions: Match to Non-human, Inconclusive, or Non-Osseous Identification. A visual decision matrix is associated with each category and specific guidelines regarding what constitutes a match to any of the three categories is provided. The triad of categories ensures that only remains that are deemed to be conclusively non-human or non-osseous are removed from accessions. If any fragment cannot conclusively be deemed non-human or non-osseous, the remains are not removed from accession and are often sent for DNA analysis to include 12S species testing.

This method of identification of non-human and non-osseous remains can easily be incorporated into any size laboratory that deals with fragmentary osseous materials. The equipment necessary for this type of analysis is relatively inexpensive and the methodology for conducting this type of histological testing is not highly complicated.

Physical Anthropology, Histology, Human/Non-Human

H71 Present State and Future Prospects of Forensic Age Estimation in Living Adolescents and Young Adults: Recommendations of the Study Group on Forensic Age Diagnostics

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After attending this presentation, the attendees will become acquainted with the spectrum of methods recommended by the Study Group on Forensic Age Diagnostics for age estimation in living adolescents and young adults. Moreover, attendees will understand the influence of the ethnicity and socioeconomic status of the individuals to be examined on the systems of characteristics studied, as well as that of diseases affecting growth and development.

This presentation will impact the forensic science community by demonstrating the complexity of forensic age estimations in living individuals. Age estimations performed *lege artis* are of great sociopolitical significance as they reinforce legal certainty.

As a result of increasing cross-border migration movements, recent years have seen a rise in numerous countries in the numbers of immigrants whose birth date is not unequivocally documented. Because of this development, forensic age estimations in living adolescents and young adults have become an integral part of forensic practice. The persons to be examined are foreigners without valid identification documents who appear to have misstated their age, which is of legal significance in criminal, civil, and asylum procedures.

The interdisciplinary Study Group on Forensic Age Diagnostics was founded in the year 2000 in Berlin, Germany. According to the recommendations of this study group, a physical examination, an X-ray examination of the hand, and a dental examination which records dentition status and evaluates an orthopantomogram should be carried out. In cases where skeletal development of the hand is complete, an additional radiological examination of the clavicles should be used.[1]

Besides anthropometric measurements such as height, weight, and constitutional type, the physical examination covers the externally recognizable signs of sexual maturity. The physical examination is of particular significance in excluding potential externally recognizable age-relevant diseases and checking whether the results of the skeletal and dental age determination are consistent with the development of the organism as a whole.

Criteria in evaluating the hand radiograph are the shape and size of the individual bone elements and the state of ossification of the epiphyseal plates.

Of particular relevance in the dental examination are the stages of eruption and mineralization of the third molars.

After skeletal development of the hand is complete, assessment of the state of ossification of the medial clavicular epiphysis plays a decisive role. Reference studies are available both for projection radiography and for computed tomography.

To increase reliability and improve the detection of age-relevant developmental disorders, all the above methods should be used. In this process, each part of the examination should be performed by a specialist with forensic experience.

As a rule, no forensically applicable reference studies are available for the regions of origin of the persons to be examined, giving rise to the question of whether serious developmental differences exist between the different ethnic groups which would preclude application of the relevant age standards to members of ethnic groups other than that of the reference population. Within the relevant age group, ethnicity has no appreciable influence on skeletal maturation. The rate of ossification is primarily dependent upon the socioeconomic status of a population. A comparatively lower socioeconomic status leads to a delay in development and, when standard reference studies are applied, to an underestimation of age. In the case of the eruption and mineralisation of the third molars, it has been determined that Black Africans manifest an accelerated development in comparison to Europeans. By contrast, in the case of Asians, a comparative retardation is to be reported. For this reason, population-specific reference studies must be used in assessing third molar development for the purposes of age estimation practice.

In the future, it is to be expected that radiation-free imaging techniques will increasingly be used for assessing skeletal maturation and tooth development. First ultrasound studies have been undertaken on ossification of the radius, pelvis, and clavicle. Furthermore, MRI studies on ossification of the radius, knee, and clavicle have been published. Most of these studies do not fulfill the requirements of forensically usable reference studies. There is a need for further research in this area.

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Age Estimation, Living Individuals, Ethnicity

H72 A Bayesian Approach to Multifactorial Age-at-Death Estimation: A Validation Study

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After attending this presentation, attendees will be able to assess the efficacy of a Bayesian multifactorial age-at-death estimation method. The goal of this presentation is to inform attendees about the performance of a Bayesian multifactorial approach to age-at-death estimation.

This presentation will impact the forensic science community by presenting a validation study of a statistically sound yet simple way to combine multiple estimates of age-at-death.

Accurate age-at-death estimation is often crucial to successful identification of unknown skeletal remains in a forensic setting. Garvin and Passalacqua showed that forensic anthropologists have a preference for age-at-death methods based on the pubic symphyses, sternal ends of the fourth ribs, and auricular surfaces, but that there is little consensus when combining estimates from multiple methods.[1] Practitioners often use their experience to combine methods and many previous techniques for multifactorial age estimation use linear combinations or simple averages of the separate age estimates and have little utility or statistical validity.

This research contributes to the validation of a Bayesian multifactorial approach first presented at the 2011 American Academy of Forensic Sciences Annual Scientific Meeting. Uhl *et al*. designed “look-

up tables" for easy use by forensic anthropologists for combining age-at-death estimates from different skeletal elements, including the pubic symphysis, auricular surface, and sternal rib ends.[2] This validation study tests the efficacy of this approach in identified cases analyzed by anthropologists at the New York City Office of Chief Medical Examiner Office. Review of case files provided data (Suchey-Brooks phases, İşcan sternal rib end phases, and Lovejoy auricular surface phases) to generate age estimates from the look-up tables. Those age estimates were compared with the actual age of the decedents for accuracy and precision.

Case files for 78 positively identified cases from the New York City Office of Chief Medical Examiner provided phase scores for pubic symphyses, sternal ends of the fourth ribs, and the auricular surfaces, as available. These scores were recorded upon initial anthropological assessment and only later were the individuals positively identified. Look-up tables first presented by Uhl *et al*. and published in Uhl provide mean log ages for each phase.[2,3] To combine the estimates, the precisions (the inverse of the mean age variance) for each indicator were summed. The inverse of the summed precisions is the within-indicator variance. The between-indicator variance is the variance of the mean log ages for the indicators, and the total variance is the sum of the within- and between-indicator variance. The standard deviation is the square root of the total variance; to obtain a 95% Confidence Interval (CI) for normally distributed data, multiply the standard deviation by 1.96 (a standard scaling variable for how wide a curve will be when normally distributed) and add and subtract that from the overall mean log age. Narrower confidence intervals were also constructed (90%, 75%, and 50%). The final step is to exponentiate (convert from log numbers to regular numbers) the endpoints of the interval and the mean log age to convert it from log years to actual years.

Overall accuracy of the point estimates is 6.45 years, while the overall precision is -4.62 years. Coverage (the percent of individuals falling within the confidence intervals) is very good. The 95% CI contained 77 of 78 individuals (99%), the 90% CI contained 75 of 78 individuals (96%), the 75% CI contained 71 of 78 individuals (91%), and the 50% CI contained 57 of 78 individuals (73%).

This Bayesian method of combining age estimates is advantageous for forensic anthropologists for many reasons. First, it is easy to use because the scoring methods are familiar. The look-up tables simplify the process of combining estimates from different age indicators, even if they are disparate. Second, it is a statistically sound means for combining separate age estimates—a method that could be demonstrated in case reports and testimony. Finally, the results of this validation indicate this method provides relatively accurate and precise age estimates. All analyses were done in R, a free statistical software program, so anthropologists can construct more population specific look-up tables for their own purposes.

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Age-at-Death, Bayesian, Multifactorial

H73 Testing the Accuracy of North American Growth Standards to Estimate Age-at-Death in Modern South African Subadults

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After attending this presentation, attendees will have knowledge of population variation in subadult growth. Long bone lengths of modern South African children are compared to North American children as a means to address validity and reliability of current methods.

This presentation will impact the forensic science community by demonstrating a need for population-specific age-at-death estimation methods from long bone lengths and the need for appropriate predictive statistical models.

The longitudinal growth study of Maresh has been adopted into anthropological literature and has been influential in estimating age-at-death from long bone lengths of modern subadults.[1] While this data has been reprinted in several research texts, the original purpose was to evaluate normal growth, not to estimate age. Though accuracy is low when used to evaluate age-at-death of modern North Americans, the accuracy of the data to estimate age-at-death is not known when applied to geographically distinct groups from North America.[2] The purpose of this study is to compare the maximum and relative diaphyseal lengths of Maresh charts to modern South African groups as a means to explore the applicability of the Maresh charts and to advocate for population-specific research.[1]

Maximum diaphyseal lengths of six long bones were recorded on 640 South African children (male and female) between birth and twelve years of age. A 21st-century sample was obtained from Lodox Statscan images from the Forensic Pathology Services in Salt River and the Red Cross War Memorial Children's Hospital in Cape Town, South Africa (n=600).[3] The 20th-century sample, which is temporally similar to Maresh, was acquired from the Raymond A. Dart Human Skeletal Collection (n=40).[1] A separate study validated the radiographic measurements were similar to dry bone measurements.[4]

Percent correct was done for both the 20th- and 21st-century South African data in order to gauge the accuracy of Maresh (1970). Results were higher for the Dart sample, with 41% correct while the 21st-century sample demonstrated 22% correct classification. The mean and relative long bone lengths of the Maresh (1970) and Lodox samples were sorted by age and bone and were compared using Student's t-tests. Using the mean values of each age group, statistically significant differences were demonstrated ($p < 0.05$) for most ages above seven years and for all long bones, except the radius. Mean differences between North Americans and South Africans revealed differences in growth. At birth, South Africans are larger than North Americans for all bones. A transition in size between groups was noted from three to seven years. At 12 years of age, South Africans were significantly smaller than North Americans for all bones. No statistically significant differences ($p>0.05$) were noted in relative long bone lengths. Similar results to mean maximum lengths were obtained when mean relative lengths were explored through graphical parameters. The mean relative lengths of all bones for the South African sample are larger than North Americans at birth, only to lag between four and nine years. By 10 years, the relative lengths begin to increase and by age 12 the proportions are similar to North Americans.

The South African and North American samples are of different socioeconomic status and genetic background, both of which contribute to differential growth. Mean adult stature in South Africans is smaller than the mean adult stature of North Americans in the 1970s. This difference in size mimics differences found in long bone lengths seen in the current study at age 12 years.

The problems with data used to estimate age-at-death are highlighted when applied to South African subadults. Due to a lack of modern skeletal material and a void of appropriate predictive methods,

validation of current subadult age-at-death techniques is difficult. Full-body Lodox Statscan images are an invaluable tool for addressing the dearth of subadult skeletal material. Data is continually being collected with the goal of developing population-specific age-at-death tables for South Africans.

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Long Bone Lengths, Relative Lengths, Radiographs

H74 Error and Uncertainty of Adult Age Estimation of the Pubic Symphysis in an Australian Sub-Population Using Computed Tomography

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After attending this presentation, attendees will gain awareness of: (1) the error and uncertainty associated with the application of the Suchey-Brooks (S-B) method of age estimation of the pubic symphysis to a contemporary Australian population; (2) the implications of sexual dimorphism and bilateral asymmetry of the pubic symphysis through preliminary geometric morphometric assessment; and, (3) the value of Three-Dimensional (3D) autopsy data acquisition for creating forensic anthropological standards.

This presentation will impact the forensic science community by demonstrating that, in the absence of demographically sound skeletal collections, postmortem autopsy data provides an exciting platform for the construction of large contemporary "virtual osteological libraries" for which forensic anthropological research can be conducted on Australian individuals. More specifically, this study assesses the applicability and accuracy of the S-B method to a contemporary adult population in Queensland, Australia, and, using a geometric morphometric approach, provides an insight to the age-related degeneration of the pubic symphysis.

Despite the prominent use of the S-B (1990) method of age estimation in forensic anthropological practice, it is subject to intrinsic limitations, with reports of differential inter-population error rates between geographical locations.[1-4] Australian forensic anthropology is constrained by a paucity of population specific standards due to a lack of repositories of documented skeletons. Consequently, in Australian casework proceedings, standards constructed from predominately American reference samples are applied to establish a biological profile. In the global era of terrorism and natural disasters, more specific population standards are required to improve the efficiency of medicolegal death investigation in Queensland.

The sample comprises Multi-Slice Computed Tomography (MSCT) scans of the pubic symphysis (slice thickness: 0.5mm, overlap: 0.1mm) on 195 individuals of Caucasian ethnicity aged 15 – 70 years. Volume rendering reconstruction of the symphyseal surface was conducted in Amira® (v.4.1) and quantitative analyses in Rapidform® XOS. The sample was divided into ten-year age subsets (e.g., 15 – 24) with a final subset of 65 – 70 years. Error with respect to the method's assigned means were analyzed on the basis of bias (directionality of error), inaccuracy (magnitude of error), and percentage correct classification of left and right symphyseal surfaces. Morphometric variables including surface area, circumference, maximum height and width of the symphyseal surface, and micro-architectural assessment of cortical and trabecular bone composition were quantified using novel automated engineering software capabilities.

The results of this study demonstrated correct age classification utilizing the mean and standard deviations of each phase of the S-B method of 80.02% and 86.18% in Australian males and females, respectively. Application of the S-B method resulted in positive biases and mean inaccuracies of 7.24 (±6.56) years for individuals less than 55 years of age, compared to negative biases and mean inaccuracies of 5.89 (±3.90) years for individuals greater than 55 years of age. Statistically significant differences between chronological and S-B mean age were demonstrated in 83.33% and 50% of the six age subsets in males and females, respectively. Asymmetry of the pubic symphysis was a frequent phenomenon with 53.33% of the Queensland population exhibiting statistically significant (χ^2-$p<0.01$) differential phase classification of left and right surfaces of the same individual. Directionality was found in bilateral asymmetry, with the right symphyseal faces being slightly older on average and providing more accurate estimates using the S-B method.[5] Morphometric analysis verified these findings, with the left surface exhibiting significantly greater circumference and surface area than the right ($p<0.05$). Morphometric analysis demonstrated an increase in maximum height and width of the surface with age, with most significant changes ($p<0.05$) occurring between the 25 – 34 and 55 – 64 year age subsets. These differences may be attributed to hormonal components linked to menopause in females and a reduction in testosterone in males. Micro-architectural analysis demonstrated degradation of cortical composition with age, with differential bone resorption between the medial, ventral, and dorsal surfaces of the pubic symphysis.

This study recommends that the S-B method be applied with caution in medicolegal death investigations of unknown skeletal remains in Queensland. Age estimation will always be accompanied by error; therefore, this study demonstrates the potential for quantitative morphometric modeling of age-related changes of the pubic symphysis as a tool for methodological refinement, providing a rigorous and robust assessment to remove the subjectivity associated with current pelvic aging methods.

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Age Estimation, Pubic Symphysis, Australia

H75 Suchey-Brooks Method Applied to 3D Symphysis: A Comparative Study on a Sample of 193 Real Pubic Bones and Their Virtual Copies

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After attending this presentation, attendees will learn how the Suchey-Brooks method of Age-At-Death (AAD) estimation can be applied to virtual bones and if virtual anthropology is reliable enough to replace traditional methods.

This presentation will impact the forensic science community by showing how classical anthropological AAD techniques can be used for analysis of virtual pubic symphysis, what are its scopes for the future, and how it can be improved to produce optimal results.

Virtual anthropology has been greatly developed by numerous teams in the past few years. Excellent agreement has been shown between the results of bone sample analysis and those of Three-Dimensional (3D) image analysis. The goal of this study was to show whether 3D reconstructions of pubic symphyses could render accurate AAD estimations by applying the Suchey-Brooks technique, in comparison to the original dry bones. A secondary objective was to test the inter-observer error between the results obtained by an experienced observer and a novice.

The study was carried out on a series of 193 dry pubic symphysis of known age and sex at death, obtained from the anthropological collection of Montpellier (Pr Baccino). Two observers, an experienced and a novice physician in forensic medicine, performed AAD estimation using the Suchey-Brooks method. The real ages of the bone samples were not known to either of the two observers prior to the test. Each observer first analyzed the series of real bones and their virtual copies afterward. The dry bones were each scanned separately on a large detector CT using high-resolution and standard mode. Helical acquisitions were obtained with 64 rows and the slice thickness was 0.5mm for acquisition and 1mm for reconstruction. Volume Rendering Technique (VRT) reconstructions were performed for each sample in order to obtain 3D images for every bone. For each observer, comparative tests were done between the AAD estimates of dry bones and the AAD estimates of 3D bones. Consecutively, inter-observer comparisons were performed. The Kappa reproducibility test was used for calculation of intra-observer and inter-observer agreement.

This study was performed on a population of 193 dry pubic symphysis (52 female and 141 male) from which 185 virtual bones (50 female and 135 male) were produced after CT. The loss of eight virtual bones was due to human error.

The study shows poor inter-observer reproducibility for the dry bones (Kappa=0.26) as well as for the scanned bones (Kappa=0.35). The intra-observer (dry bones versus scanned bones) Kappa coefficients are of 0.28 for the novice (poor reproducibility) and 0.44 for the experienced observer (moderate reproducibility). This suggests that the Suchey-Brooks method is better applied by a more experienced observer. The mean difference between real age and estimated age for each of the 193 dry bones varies between -0.4 years and +3.4 years, whereas for virtual bones, the mean difference on the 185 subjects was between +1.66 years and +2.76 years.

The poor inter-observer reproducibility could be explained by the different levels of experience of the two readers. Indeed, there is moderate intra-observer reproducibility with the more experienced physician, whereas, this value is poor with the novice. It is also observed that the mean difference between real bone ages and estimated bone ages is not greater than 3.4 years. This shows that the age estimations are highly reliable not only with dry bones, but also with virtual bones.

The application of CT to virtual anthropology is promising for the future. CT parameters need to be clearly defined and improved to obtain better reconstructions. In the study, the parameters were thoroughly tested so as to produce the most optimal visual results using VRT. The 3D reconstructions were of very high quality, but they can probably be refined. It should be kept in mind, however, that no matter how good the quality of a 3D image is, it remains virtual and, therefore, different from a real dry bone or a cast. It would be interesting to redefine the existing anthropological AAD techniques in order to make them applicable to virtual bones.

Suchey-Brooks, Virtual Anthropology, Pubic Symphysis

H76 Investigating Macroscopic Changes of the Pubic Symphysis at the Young End of the Age Spectrum

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After attending this presentation, attendees will have been provided with an introduction to an aging method using the pubic symphysis that provides more precise estimates than are currently assigned to individuals under the age of 40 years.

This presentation will impact the forensic science community by systematically documenting age-related changes that occur at the pubic symphysis within the first two decades after sexual maturity and by providing a method with more accurate and precise age estimates for individuals at the younger end of the age spectrum.

Among the standards employed for age estimation in forensic contexts, the Suchey-Brooks pubic symphysis method is a highly favored technique.[1,2] Difficulties arise, however, when researchers are forced to assign phase-based ages to complex patterns of growth and development *and* degenerative changes associated with biomechanical loading, both of which can be highly variable among individuals and across populations. In regard to the pubic symphysis, it has been documented that minute morphological transitions occur along the ventral and dorsal borders and at the superior and inferior aspects of the face and pubic tubercle.[1,3-7] Current pubic symphyseal aging methods combine the morphology associated with the developmental changes that occur into the mid-30s with the degenerative changes that span the latter portion of the age spectrum. The most popular methods are phase-based; however, the definitions currently used to assign age intervals may not be adequately defined and/or accurately understood by burgeoning researchers and seasoned practitioners alike. Furthermore, phase-based systems assume that these complex changes occur in lock-step, which may not accurately reflect biological reality.

This study investigated macroscopic changes in forensically relevant modern U.S. samples of known age, sex, and ancestry from the Maricopa County Forensic Science Center in Phoenix, Arizona, as well as donated individuals from the William M. Bass Forensic and Donated Collections at the University of Tennessee, Knoxville (n=210). As disparities have been noted in the Hispanic population with pubic symphyseal aging, the present sample is comprised of American Black and White individuals only.[8] Age-related traits at locations with ontogenetic and biomechanical relevance were broken into components and scored. The components included the pubic tubercle, the superior apex of the face, the ventral and dorsal demifaces, and the ventral and dorsal symphyseal margins. Males and females were scored and analyzed separately (n=141 and n=69, respectively). A range of two to three categorical values were assigned based on the changes observed within each component. Scores for each category ranged from presence/absence to a three-step categorical option, thus allowing for simplistic and unambiguous scoring. Transition analysis was applied to elucidate the transition ages between the morphological states of each component. The categorical scores and transition analysis ages were subjected to multinomial logistic regression to derive estimates with high classification accuracy. In addition, inter-observer error was assessed on 100 pubic symphyses using a kappa statistic.

The transition ages for the categorical states generally agree with the documented literature.[7] There were significant sex differences (α=.05) in the ages at transition for all categorical traits except the pubic tubercle, with males transitioning from one state to the next at earlier ages than females (1 – 5 years earlier). Interestingly, this is contrary to the expectation with regard to developmental traits, where females are usually precocious. The categorical output gave smaller age ranges for individuals aged 18 to 40 years, which translated to minimum ranges of three years. The logistic regression provided high classification rates for the age categories, with classification accuracy reaching 93%; the predictive model was significant (p<.001). Inter-rater agreement was high for the pubic tubercle, superior apex, ventral demiface, and ventral rampart (kappa≥0.8), but lower for the dorsal margin (0.6) and dorsal demiface (0.5). The latter will be addressed, with possible revision of the scoring categories and/or definitions.

Based on these preliminary results, it is proposed that an unambiguous scoring method for age-related changes of the pubic symphysis can provide precise age estimates for younger adults, and thus strengthen the quantification of the methods employed when building a biological profile for forensically relevant remains. This method can be combined with other methods for age estimation in young adults (i.e., sacrum and clavicle) or used as a stand-alone method to derive more precise and accurate estimates from the pubic symphysis than is possible with existing methods.

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Age Estimation, Pubic Symphysis, Logistic Regression

H77 A Validation Study of Computed Tomography Extracted Cranial Landmarks

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After attending this presentation, attendees will understand the utility of Computed Tomography (CT) cranial scans for 3D geometric morphometric analysis, positive identifications, and ancestry estimation.

This presentation will impact the forensic science community by presenting the utility of using extracted cranial landmarks from CT scans that could be used in the estimation of ancestry.

The main research objective of this study was to investigate the variation between cranial landmarks extracted from CT scans and those collected from digitization. The sample consisted of 29 individuals from the Morton collection housed at the University of Pennsylvania's Museum of Archaeology and Anthropology. The dry skulls were digitized with a 3D digitizer and scanned with CT. The CT scans were made available by Janet Monge as part of the Open Research Scan Archive (ORSA) at the University of Pennsylvania. Thirteen traditional cranial landmarks were obtainable from each CT scan using the coordinate option of a software program. These coordinates were then compared to the digitized coordinates. A geometric morphometrics software program was used for multivariate statistical analysis. Procrustes' superimposition was used to translate, scale, and rotate the landmark data until a consensus configuration was identified with a least-square fit. New shape coordinates were derived for the entire dataset and for each digitized and scanned individual.

The digitized landmarks and CT-extracted coordinates were first treated as two separate groups to examine for overall differences. Principal Components Analysis (PCA) was performed to reduce dimensionality by decreasing the number of variables to the few that represented the majority of variation. Most of the variation (86%) was in the first four principal components with 57% of the variation found in the anterior portion of the cranium along the first principal component. Canonical Variant Analysis (CVA) was used to identify the landmarks responsible for the variation between the CT coordinates and the digitized coordinates. CVA provided one significant canonical variant, which accounted for 99.8% of the variation and included landmarks along the midline (e.g., nasion and opisthion). A Discriminant Function Analysis (DFA) was also performed to discern the approximate level of separation between the two groups with a correct classification rate of 64% using cross-validation. This was expected and illustrated the similarities between the two groups of coordinates. In addition, a Multivariate Analysis Of Variance (MANOVA) was performed to measure the variance between the two groups. A significant difference was found between the CT and the direct coordinates (*p-value=0.022)*.

To directly assess the difference between digitized and CT coordinates of each individual cranium a Procrustes' superimposition, PCA, and Procrustes' ANOVA were performed using a geometric morphometrics software program. Average Procrustes' ANOVA results of the Procrustes' coordinates suggested that the CT and digitized coordinates of each individual were not significantly different in terms of shape (*p-value=0.578)*. Also, bilateral variables were insignificant (*p-value=0.340*). The greatest PCA-derived one principal component variation was found in bilateral landmarks (e.g., zygomaxillare), which illustrated some distortion occurring in the CT coordinates for bilateral landmarks. Overall, these results validated the utility of CT coordinates.

The results of this study showed significant differences between CT and digitized coordinates when used in large datasets. Most of the variation observed included landmarks along the midline between the two groups. In contrast, the individual analyses exhibited the largest variation among bilateral landmarks. The significant differences found for the entire dataset suggested that the combination of CT and digitized coordinates may not be appropriate for population variation studies. However, the individual results of this study supported the utility of CT coordinates for putative identifications.

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Geometric Morphometrics, Computed Tomography, Crania

H78 Molar Size and Shape in the Estimation of Biological Affinity: A Comparison of Relative Cusp Location Using Geometric Morphometrics and Interlandmark Distances

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After attending this presentation, attendees will have gained knowledge on the variation present in the size and shape of molars between ancestral groups. Furthermore, attendees will learn which cusp measurements can be used to accurately estimate ancestry.

This presentation will impact the forensic science community by contributing to the understanding of modern human variation in molar shape and size, which can, in turn, be used to aid in the estimation of ancestry in unidentified individuals encountered in forensic casework.

Dental morphology has long been utilized in anthropology as a means to examine biological affinity.[1-3] Dental variation has traditionally been recorded in accordance with the Arizona State University Dental Anthropology System (ASUDAS) scoring procedures in which morphological traits were scored either by presence or absence or by degree of expression on an ordinal scale.[4] Morris' work on occlusal polygons represented the first use of geometric techniques to analyze cusp location on molars for the estimation of biological affinity in modern human populations.[5] Occlusal polygons utilize the length, angles between cusp tips, and area of the molar crowns by using each cusp tip as a vertex. Occlusal polygons have continued to be utilized by Bailey and Bailey et al. as a means to investigate biological affinity, but occlusal polygons have also been analyzed using updated geometric morphometric approaches.[6-11] The purpose of this research was to analyze the variation present in the relative cusp location in two modern population groups and to assess whether this variation can be used to accurately discern ancestry.

Coordinate data were collected from the cusp tips of 216 adult upper and lower first and second molars. The sample consisted of 107 (F=52, M=55) American Blacks from the Hamann-Todd Osteological Collection at the Cleveland Museum of Natural History and of 109 dental casts from American Whites (F=60, M=49) housed at the University of Alaska Fairbanks, Department of Anthropology. Using a coordinate digitizer, the tip of the stylus was placed on the apex of each cusp tip. In the event of slight wearing, the stylus was placed at the center of the exposed dentine. Only the x and y coordinates were retained for the analyses because the differential degree of wear between specimens would introduce noise to the data along the z-axis. The coordinate data for each molar were analyzed individually through MorphoJ to perform a Generalized Procrustes Analysis (GPA) and calculate centroid size.[12] The GPA translated, scaled, and rotated each specimen to a common coordinate system so that each landmark's coordinates could be directly compared independent of size. The Procrustes coordinates were exported and analyzed using FORDISC 3.0 custom database feature to perform discriminant function analysis (DFA).[13] Lastly, interlandmark distances were calculated from the coordinate data and also analyzed through DFA. All DFA utilized leave-one-out cross-validation and forward stepwise selection of variables.

The results of the two-way DFA between Blacks and Whites using the Procrustes coordinates ranged from 70% – 85% total correct classification. Correct classification using Procrustes coordinates for Blacks ranged from 65% – 82% while correct classifications for Whites ranged from 74% – 87%. In each analysis, Whites classified correctly more frequently than Blacks. The analysis that yielded the highest classification accuracies was a combination of the first lower molar and the second upper molar (B=82%, W=87%; total correct=85%). The results of the DFA on the interlandmark distances ranged from 62% – 80% correct classification. The analysis that resulted in the highest correct classification of interlandmark distances utilized the upper first molar and both lower molars. Most of the interlandmark distances selected for discrimination between White and Black groups included the distal aspect of the tooth, frequently involving the hypocone, for the upper molars, and the hypoconulid for the lower molars.

The molars, especially when used in combination with one another, can accurately differentiate ancestral affiliation between American Whites and Blacks and can be used to estimate ancestry in unknown forensic cases with up to 80% correct classification. While the shape variables derived through the GPA yielded slightly higher correct classifications, interlandmark distances also showed promise as a means to determine biological affinity and can be more easily measured by using standard calipers.

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Molar Morphology, DFA, Ancestry Estimation

H79 The Sagittal Suture of the Cranial Vault in Korean

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WITHDRAWN

H80 Received Wisdom vs. Reality: Utilizing Amelogenin Profiles to Evaluate the Accuracy of Skeletal Morphologic and Metric Sex Assessments in Adults

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After attending this presentation, attendees will gain a further appreciation of how genetic data can enrich the field of forensic anthropology by providing an independent line of evidence for the assessment of sex.

This presentation will impact the forensic science community by providing one method to evaluate the accuracy of morphologic and metric assessment of sex from skeletal remains.

Correct sex assessment is arguably the most important determination that is rendered upon unidentified human skeletal remains because most missing person databases are organized into males and females. Thus, to assess biologic sex incorrectly can drastically reduce the chance for identification. In addition, if sex is erroneously assessed, subsequent age and stature estimates will also likely be in error. Forensic anthropologists routinely utilize morphologic and metric analyses to assess sex from the skeleton, but exactly how accurate is any individual practitioner? The received wisdom is, in part, based upon forensic anthropology pioneers T.D. Stewart and W.M. Krogman stating they each could assess sex with 90% to 100% accuracy utilizing the entire skeleton and 80% to 92% utilizing the skull alone. Although these estimates are individual accuracy rates, the implication was that with enough practice, these percentages could be attained by other forensic anthropologists. Obviously, this is flawed logic and in these days of *Daubert* and error rate issues, individual forensic anthropologists should know their own error rate. Historically, the only way for the forensic anthropologist to learn the accuracy of a sex assessment was through identification. Thus, errors were likely under-reported. Today, we have another tool to reveal these errors: the Amelogenin locus that is a part of many DNA analyses.

The medical examiner's office has submitted more than 1,000 postmortem tissue samples for DNA analyses. Amelogenin profiles were derived for 862 cases and skeletal sex assessments were performed on 840 of these cases, with 350 of these being performed by the author. Thus, for each of these 350 sex assessments, there now exists a genetic line of evidence that can be used as a source of comparison and evaluation. While genetic data are not infallible, these Amelogenin profiles are a powerful tool for such an evaluation.

After controlling for variables that can influence a skeletal sex assessment, these 350 individual cases were subdivided by condition and completeness into categories ranging from a full body to a single osseous element. Because accuracy can be affected by incompleteness, different rates are expected and discussed. Additional sources of potential bias, such as the presence of soft tissue, personal effects, and name associations are also utilized to pare down the number of cases to minimize bias. A total of 203 cases involved skeletonized remains with no appreciable soft tissue or other associated factors. This present research will demonstrate that when all known biases are minimized, such as can be expected when only a cranium is present for assessment, a truer accuracy rate for skeletal assessment may be attained. However, if the error rate becomes unacceptably high, the recommendation is made to not render a sex assessment.

Of particular interest are sex assessments from the cranium alone. More errors were made when the cranium alone was relied upon than any other skeletal element or set of elements. Almost all of these errors were in the same direction: misclassifying small males as females. One explanation for this is that the majority of the skeletal analyses were performed upon foreign national migrants, most of whom are small males. Other errors were made on single elements, such as a femur, so one question to ask is, "Why render an assessment in all instances?" One reason, albeit poor, for stretching the limits of sex assessment methods is to choose one of two database categories: John Doe vs. Jane Doe.

Forensic anthropologists stand on the shoulders of giants. Today, the field is better for at least two reasons: anthropologists have learned from mentors and have developed methods that can test pronouncements. Observation and experience founded this field, and additional research and education are refining it. A part of this refinement is knowing one's own error rates for the different methods employed. After determining these rates, limitations can then be set on what skeletal elements should be used in sex assessments. Many forensic anthropologists chose this field because our assessments can be proven wrong or validated. DNA results enrich our field. Amelogenin profiles should be viewed as an independent line of evidence to evaluate skeletal sex assessments and should be welcomed. Other new tools, such as the National Missing and Unidentified Persons System (NamUs), now enable entry of the sex assessment of *Undetermined* because program algorithms will search all missing person lists, regardless of sex.

Forensic Anthropology, Sex Assessments, Amelogenin Locus

H81 Current Practices in Forensic Anthropology for Sex Estimation in Unidentified Adults

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After attending this presentation, attendees will be presented with an analysis of the methods currently employed by forensic anthropologists to estimate the sex of an unknown adult individual, and the current practices for reporting results when multiple methods are employed. This research is part of a larger study analyzing the same information within all of physical anthropology, including skeletal biology, and bioarchaeology, in addition to forensic anthropology.

This presentation will impact the forensic science community by reporting the variation within the field for sex estimation from the human skeleton. The goal of this research is to disseminate the results of a questionnaire among practitioners to promote discussion and standardization. Understanding the degree or variability, method preference, and modes of reporting is the first step toward standardization within the field of forensic anthropology for sex estimation practices.

Estimation of sex is generally the first step when constructing the biological profile of an unidentified individual, primarily because most of the methods currently used for ancestry, stature, and age estimation are sex-specific. Current methods used to estimate sex consist of either quantitative measures of skeletal elements or qualitative observations of gross morphology, primarily of the pelvis and skull. Historically, morphological assessments dominated forensic anthropology, especially for sex and ancestry estimation; however, there has been a shift in the past several decades toward the development of quantitative approaches.[1] Furthermore, attempts have been made to standardize the various methods used for biological profile estimation and the data collection process, including Buikstra's and Ubelaker's work *Standards for Data Collection from Human Remains*, best practice recommendations for sex assessment by the Scientific Working Group for Forensic Anthropology, and more recently with the Osteoware Standardized Skeletal Documentation Software.[2-4] Despite attempts for standardization, the methods employed and the way in which the results are reported for biological profile parameters varies considerably by practitioner. In many forensic cases, both metric and morphological methods are employed for sex estimation to generate the biological profile. Inconsistency within forensic anthropology for sex estimation method preferences, method applications, and subsequent result reporting is problematic in light of the *Daubert* decision and raises questions of methodological protocol for sex estimation and consistency within the field.[5]

An electronic questionnaire was created and participants were recruited via email through a bulk list server distribution from professional organizations that included forensic anthropologists or

through announcement of the research on these organizations' websites. The online questionnaire consisted of 32 questions concerning the participant's education, background, and their preferences and practices for sex estimation. Like Garvin's and Passalacqua's research on current age-at-death estimation practices, a multiple choice format was utilized when possible, and for the methodological questions, a rank system was used and included an area for additional written comments.[6] The survey software double-blinded all identification information, so that participation in the survey was anonymous on all accounts and participants were not compensated for taking part in this research.

Responses were received from 152 individuals; however, for this component of the research, only the individuals who self identified as forensic anthropologists were analyzed (n=92). The pelvis was overwhelmingly preferred as the best indicator of sex (94.3% selected as first choice), followed by use of the skull (64.8% selected as second choice), and then by the long bones as the third preferred area (65.1% selected as third choice). Using qualitative measures, traits listed in *Standards* were ranked highest for the skull and for the pelvis the three traits of Phenice ranked highest. For metric assessment, FORDISC was the preferred method for the skull and postcrania.[7-8] Most practitioners (63.6%) prefer to use both qualitative and quantitative methods; however, when both are not used, qualitative methods (23.9%) were preferred nearly twice as much as metric methods (12.5%). Finally, when results from the multiple methods employed disagreed, 41.2% of practitioners presented the results of all methods, 20.0% gave preference to one skeletal area over the results from others, and 15.3% decided which assessment to assign the individual based on experience and the overall impression. These findings highlight the high degree of variability in the methods used for sex estimation and the need for standardization within the field.

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Sex Estimation, Biological Profile, Standardization

H82 The Problem of Estimating Sex From the Skull: A Comparison of Methods Applied to a Modern Colombian Sample

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The goal of this presentation is to demonstrate that metric methods of sex estimation from the skull do not yield classification accuracies of over 80% in a modern Colombian sample while visual methods applied to the same population perform slightly higher.

This presentation will impact the forensic science community by suggesting that forensic anthropologists utilize skeletal elements other than cranial remains for estimating sex. In cases when only cranial remains are present, caution should be exercised in arriving at sex estimates, particularly in cases where no population-specific standards are available.

In forensic anthropological contexts, estimating the biological profile from skeletal remains is of paramount importance. Researchers agree that elements of the bony pelvis provide the most reliable sex estimates, typically from visual assessment. Until recently, in cases where pelvic elements were absent or too damaged to be of use, protocols historically called for workers to preferentially choose the skull, as it was considered the second-best sex indicator. Recent publications in the literature have challenged this notion and have convincingly argued that post-cranial elements are much more sexually dimorphic than the skull. While this shift has been embraced in the United States, forensic anthropologists working in diverse international contexts often encounter solitary crania or work in locales where population-specific criteria for sexing post-cranial remains are non-existent. As a result, the skull is often still considered as a reliable indicator of sex despite evidence to the contrary. This is particularly true in Colombia where practitioners are currently in the process of identifying scores of unidentified skeletons while simultaneously developing population-specific standards for the Colombian population. Given this reality, there is a strong need for researchers to understand which methods for estimating sex from cranial remains are suitable and which might be appropriately abandoned in Colombia.

The influential work of Giles and Eliot ushered in an era of intense interest on quantifying sexual dimorphism from metric analyses of cranial remains.[1] Typical approaches utilized discriminant function analysis of interlandmark craniometric distances for sex estimates. Many of these studies used skeletal collections derived from the late 19th and early 20th-centuries to generate standards that were utilized by forensic anthropologists around the world. During this influential time period, the work of Acsadi and Nemeskeri also brought attention to five non-metric cranial characteristics that could be used to visually assess sex (i.e., nuchal crest, mastoid process, glabellar prominence, supraorbital margin, and mental eminence).[2] These morphoscopic traits are well known today and have been reproduced in numerous protocols which have been adopted by both forensic anthropologists and bioarchaeologists.

Though the work of Spradley and Jantz convincingly argued that practitioners should shift their attention to post-cranial sex estimation, more work remains to be done on modern samples drawn from populations outside of the United States.[3] The work presented in this study was based on an analysis of cranial remains from 198 individuals (m=133; f=65) drawn from two modern Colombian reference collections currently curated in Bogotá, Colombia, and Medellin, Colombia. The pooled samples' average age-at-death was 45.8 years for males and 56.79 for females. Each cranium was digitized with a Microscribe digitizer interfaced with the software program 3Skull. Sixteen interlandmark distances mined from 3Skull were then used to calculate univariate sectioning points in order to determine classification accuracies of each interlandmark distance. In addition, a subset of the Bogotá sample was visually assessed and five morphoscopic traits were recorded blind to real sex.

Classification accuracies of the 16 interlandmark distances ranged from 55% – 77%. Of all interlandmark distances, bizygomatic breadth (ZYB) achieved the highest classification rate, a result mirroring findings of Spradley and Jantz.[3] In the case of visual assessment, the observer estimated sex based on the *gestalt* of morphoscopic traits. Overall, 81.8% of the Bogotá subsample was sexed correctly. This result mirrors the work of others who have achieved classification accuracies of over 80% from visual assessment of cranial remains.[4] Ultimately, in both this study and that of Spradley and Jantz analyses of craniometric dimensions indicate that other areas of the skeleton should be preferentially chosen for sex estimation and that in cases of isolated crania, workers must qualify the limitations of currently available methods.[3]

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Sex Estimation, Colombia, Skull

H83 A Test of Formulas Used to Estimate Stature in Modern Chileans

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After attending this presentation, attendees will understand some of the problems with previously reported stature equations developed to aid identification efforts for the "disappeared" from Pinochet-era and modern medicolegal cases in Chile.

This presentation will impact the forensic science community by providing new and more accurate equations for estimating the statures of modern Chileans.

Populations throughout history have differed in body dimensions. Modern populations are not exempt from normal variation in biological traits, both within and between various groups. Anthropologists have attempted to quantify this variability, and standards used to measure traits such as stature have been created for European and North American populations. Standards derived from European and American populations have produced mixed results when applied to Latin American skeletal samples, where there is a lack of local data that can be used to determine ancestry, sex, and stature. Creating new stature estimation equations has become increasingly important for countries such as Chile, where a legacy of human rights violations has resulted in unidentified remains.

In Chile, quantifying population variability has been difficult since living stature is not regularly recorded on official documents. To aid the Chilean government in this matter, Ross and Manneschi created equations using substituted population means from studies conducted in the 1970s and used long bone lengths from North American populations to calibrate these equations.[1] As long as these population means are similar to those of the Chilean target skeletal population, they should be effective substitutes. However, the obtained population parameters (means, variances, and shapes of the variance distributions) for these other reference groups do not match the values for local Chilean populations, introducing systematic errors when estimating stature.

This study tests Ross' and Manneschi's previously-published Chilean stature equations using a new sample of 34 males from the Cementario General Collection at the Universidad de Chile and modern forensic cases from the Servicio Medico Legal in Santiago, Chile. In lieu of recorded living statures, skeletal height was calculated for each specimen using Raxter's Revised Fully's Anatomical Method.[2] These data were substituted into Ross' and Manneschi's and Trotter's published equations for the femur and tibia, and success was evaluated using mean error statistics (inaccuracy and bias).[3] In addition, new equations were generated for comparison.

Mean stature for the Chilean test sample (161cm) is significantly different from the mean values used by Ross and Manneschi (170cm and 174cm). Mean femur length for the Chilean test sample (43.29cm) is also significantly different from the mean values used by Ross and Manneschi (46.91cm). Mean tibia length does not follow the same pattern and the values obtained for the current study (36.40cm) are not statistically different than those used by Ross and Manneschi (36.85cm). Trotter's equations for the femur and tibia perform reasonably well (inaccuracies=3.69cm and 8.59cm, respectively). However, Ross' and Manneschi's equation for the femur does not perform well, with the majority of specimens being severely underestimated (inaccuracy=20.28cm, bias=-20.78cm). Ross' equation for the tibia performs even worse, with some specimens falling well outside of Ross' published error intervals (inaccuracy=43.51cm, bias=-43.51cm). New stature equations generated from the current sample have inaccuracy values of 2.86cm (femur) and 2.31cm (tibia).

North American stature data does not appear to be an adequate proxy for modern Chileans. Researchers should avoid the temptation to substitute data from other populations when documentation of local skeletal samples is incomplete. Although limited data is available, this study emphasizes the importance of collecting all the data required to create accurate and reliable regression equations.

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Stature, Regression, Chile

H84 Sexual Dimorphism in Thai Postcranial Measurements

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After attending this presentation, attendees will understand the methods associated with sexing the skeleton, the different levels of sexual dimorphism in the skeleton and also in different populations around the world, and an example of the importance of selecting the most valid methods for the level of dimorphism.

This presentation will impact the forensic science community by raising awareness of the different levels of accuracy of sex estimation methods for different populations, and the importance of population-specific validation of metric and nonmetric methods in responsible biological profile assessment.

Sexual dimorphism has been observed and recorded in populations around the world for over a century, with recognition that different groups show different levels of sexual dimorphism. Nevertheless, forensic anthropologists universally apply nonmetric methods that focus on particular traits of the cranium and innominate for estimating sex of unknown individuals, regardless of suspected ancestry.[1,2] Furthermore, multiple methods are expected to yield equivalent levels of sexual dimorphism for a particular individual. Consequently, adherence to the *Daubert* standard of method validation is undermined by a lack of population-specific error rates in sex estimation methods.[3]

Recent published and unpublished results have indicated low degrees of sexual dimorphism in Thai crania, and moreover, typical sex markers of the postcranial skeleton, such as the pubis, appear to be less sexually dimorphic than contemporary American populations.[4] In contrast, high levels of sexual dimorphism have been observed in the postcranial skeleton of Thai in the examination of single bones (femur, tibia, and humerus), despite a generally smaller physique compared to other East Asian populations.[5] Therefore, it is necessary to compare the extent and location of dimorphism in the entire Thai postcranial skeleton as a potential alternative for biological profile assessment in which the traditional Walker and Phenice markers are less valid.

In this study, a modern sample of 122 males and 78 females from Khon Kaen University, Thailand, were compared to modern American Whites and Blacks in the Forensic Data Bank, with the objectives of testing the classification accuracy of postcranial metrics using Discriminant Function Analysis (DFA), as well as to identify the most dimorphic skeletal traits in each group. Each ancestral group was analyzed independently for seven robusticity indicators (breadth measurements) of the humerus, femur, and tibia, and for seven length measurements of the clavicle, humerus, radius, femur, and tibia, using discriminant function analysis in FORDISC 3.1 and R statistical software

programs. Forward stepwise selection was conducted to determine which measurements were most effective for differentiating sex in each ancestral group.[6,7] In all groups, robusticity indicators outperformed length measurements, achieving classification accuracies of 95.0% (White), 95.6% (Thai), and 97.8% (Black), compared with 92.6% (White), 93.0% (Thai), and 95.3% (Black). When all 14 measurements were combined, robusticity indicators continued to be more valuable characters for estimating sex.

When stepwise selection was used, classification accuracies were similar in all groups, exceeding 90%, but the Thai groups required five measurements for distinguishing males and females, in contrast to two measurements that were selected for each of American Blacks and Whites. Thus, Thai males and females can be classified with similarly high rates of accuracy, but sexual dimorphism is more pronounced in American Blacks and Whites.

Sexual dimorphism in the Thai illustrates the differential accuracy of sex estimation methods in different populations. Although the results indicate that dimorphism may be lower in Thais than in American Whites or Blacks, postcranial metrics can still be used to estimate sex accurately, in contrast to the typical nonmetric methods. In the wake of *Daubert*, the Thai sample emphasizes the importance of utilizing the most reliable and valid method in assessing parameters of biological profile.

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Sex Estimation, Postcranial Metrics, Method Validation

H85 Sex Estimation in a Modern Thai Sample Using Non-Metric Traits of the Innominate

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After attending this presentation, attendees will understand that a popular non-metric method using traits from the innominate cannot be applied reliably to other ancestries.

This presentation will impact the forensic science community by demonstrating that any sex estimation techniques must be used with caution and that the source and nature of the samples must be taken into account.

Sex estimation is a vital part of constructing the biological profile of an unknown individual. Following the *Daubert* decision (*Daubert vs. Merrell Dow Pharmaceuticals, Inc.* 1993), in order to be considered admissible in court, scientific conclusions must be based on reliable and valid methods with estimated error rates.[1] Not all methods used to construct a biological profile that are derived from one group or population are necessarily applicable to another group, and should be tested for external validity, as has been repeatedly demonstrated in metric studies of the cranium and post-cranium. This study examines how well a recent method published by Klales *et al*. (2012), which used modified Phenice (1969) traits of the innominate, performs when applied to a sample of modern Thai individuals.[2,3] The three characteristics of the innominate (medial aspect, ventral arc, and subpubic concavity) were scored on 76 Thai individuals from Khon Kaen University Hospital and were compared to the scores of modern Americans in the UTK Forensic Anthropology Center. Because the observations were recorded as ordinal data, the American and Thai groups were compared using several non-parametric tests including the Shapiro-Wilkes test, Wilcoxon signed-rank test, and the Freeman-Halton test. The Thai individuals were classified using logistic regression and linear discriminant function analysis. Statistical analyses were conducted using R (R Development Core Team 2012) and FORDISC 3 (Jantz and Ousley 2010).[4,5] In group comparisons, the modern Thai sample was found to have significantly lower scores compared to the modern American sample in all three traits. Using the discriminant function analysis and logistic regression equations developed by Klales et al. (2012) based on a modern American sample, the modern Thai sample classified very poorly: the correct classification rates of the modern Thai males were as low as 27% using the logistic regression and only 43% using discriminant function analysis.[2] The Thai females were classified more accurately, indicating that the overall scores of both the males and females are lower (more feminine) than those of the modern Americans. However, sex estimation equations derived from the Thai sample itself also showed low accuracy rates, indicating they are simply less sexually dimorphic in these traits than the American groups. Thus, the Thai sample is both more feminine and less sexually dimorphic in these traits than either the Phenice (1969) or the Klales et al. (2012) samples.[2,3] Therefore, these traits must be thoroughly tested before being applied to any other group or population other than the American White and Black from which the equations were derived.

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Non-Metric, Sex Estimation, Thai

H86 Date of Birth Estimation of Dead Bodies—A Compilation of C^{14} Reference Levels in Enamel to Assist in Identification Work

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After attending this presentation, attendees will understand how dental enamel formed after 1955 can be dated using the radiocarbon bomb-pulse. They will also learn how aggregated reference radiocarbon data can improve the precision of date-of-birth estimation. Further, the

attendees will learn that stable isotopes, aspartic acid racemization analysis, and DNA analysis of teeth can help to identify origin and sex.

This presentation will impact the forensic science community by showing how reported data are important to forensic investigators involved in identification work, since bomb-pulse ^{14}C dating can provide accurate information regarding the date of birth of unknown individuals.

For identification of unknown decedents, dental X-rays and DNA comparisons are the most important methods to determine identity, but each process requires an idea of who the deceased person is. In cases where there are no clues about the identity, then the sex, the date of birth, and age at death are decisive in the identification casework to limit and, hence, facilitate the search for possible matches. During the cold war, above-ground test detonations of nuclear weapons resulted in a substantial increase of ^{14}C in the atmosphere. By measuring such bomb-pulse-generated ^{14}C in modern biological material, a fingerprint of the time for its formation can be obtained. This method has been used in combination with aspartic acid racemization analysis (which gives an estimate of the age at death) to also estimate the year of death. Furthermore, stable isotope levels in teeth have been used to predict the origin of the person.

In this study, ^{14}C, ^{13}C, and ^{18}O concentrations in teeth from people in Mexico, the U.S., and Canada were analyzed. In order to isolate the enamel, the crown was treated with strong NaOH and sonicated. The teeth were then etched with HCl to limit external contamination. The concentration of ^{14}C in the samples was determined by accelerator mass spectrometry. DNA, ^{13}C, and ^{18}O levels in these teeth were analyzed using the Identifiler kit from Applied Biosystems, and isotope ratio mass spectrometry, respectively. Finally, all enamel ^{14}C results in this and previous studies were compiled to produce a tooth ^{14}C reference table.

Results-identified differences in ^{13}C concentrations in teeth over a limited geographical area such as North America are substantial. Teeth from Mexican subjects showed much higher ^{13}C levels than teeth from U.S. and Canadian subjects. The ^{18}O concentrations in tooth roots of U.S. subjects paralleled the levels in drinking water in the areas they were raised. Carbon-14 predicted birth year with an average absolute error of 1.8 ± 1.3 years. By using reference data on enamel ^{14}C levels, estimates of a person's date of birth, and thus age at death, can be provided with greater accuracy than that provided via radiographic analysis of enamel formation. By analysis of ^{14}C in both enamel and root of the same tooth, it could be determined whether the ^{14}C results corresponded to the rising or falling part of the bomb-pulse curve. DNA analysis of the tooth roots indicated the correct sex for all subjects tested.

In order to limit the search for possible matches, the year of birth, the year of death, sex, and geographic origin constitute important variables. Regarding age estimation, anthropological methods often show estimates of +/- 10 years, and such information will not reduce the number of possible matches significantly. In contrast, the bomb-pulse ^{14}C method provides a more accurate age by estimating the year of birth. Interestingly, substantial geographical differences of the stable isotopes ^{13}C and ^{18}O were observed, which can help indicate origin of subjects who are found dead far from their residential area. Both isotopes have also shown to display geographical variation in hair collected from only a few years back, which supports the notion that analysis of these stable isotopes in teeth are likely to be useful during many years to come. Bomb-pulse ^{14}C analysis is a robust method that can provide a very good estimate of year of birth. ^{13}C and ^{18}O analysis may give clues to geographical origin. DNA analysis can, in addition to providing an individual profile, determine the sex. All analyses described above can be performed on a single tooth.

C^{14}, Identification, Teeth

H87 Decomposition in Central Texas and Utility of a Universal Postmortem Interval

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After attending this presentation, attendees will understand the process of decomposition in Central Texas, the utility and accuracy of a universal Postmortem Interval (PMI) formula, and will be presented with a baseline postmortem interval for Central Texas.

This presentation will impact the forensic science community and medicolegal professionals by detailing the decomposition process that is unique to Central Texas with detailed explanations of each stage of decomposition, accompanied by photographic aids. This presentation will also impact the forensic science community by analyzing the pros and cons of using a universal PMI formula.[1]

Although decomposition has been studied by many over the past few decades, most notably Galloway *et al*. and Megyesi *et al*., the majority of research conducted regarding the decomposition sequence have used animals as human analogs.[2-5] These previous studies have provided a framework for further studies; however, animals are not always an appropriate proxy for human subjects.[6] Additionally, previous studies of decomposition in Central Texas had relatively small sample sizes, leaving room for potential error.[7-8] Unlike previous studies, this study includes over 60 individuals donated to the Forensic Anthropology Center at Texas State University-San Marcos. This study will provide individuals with access to a large-sample, region-specific collection of decomposition data.

Photos were collected daily for the first month after placement of each individual at the Forensic Anthropology Research Facility (FARF), and bi-weekly thereafter until decomposition was complete. Each donation was analyzed and the date of each stage of decomposition recorded. Stages of decomposition recorded at the facility were: fresh, early decomposition, late decomposition, mummification, and skeletonization.[2] In this study, fresh describes the conditions of the bodies at time of placement, prior to decomposition. Early decomposition at the facility is characterized by marbling, bloat, maggot activity, skin slippage, and increased odor. Donations at the FARF show that early decomposition occurs between 2 and 13 days after placement (34-302 ADD). Advanced decomposition is characterized by the loss of marbling, a sunken stomach due to the purge of fluid and gases from the body, and decreased insect activity. Advanced decomposition is initially shown to occur between 6 and 53 days after placement (212.5-784.4 ADD). Mummification occurs when all fluid has left the body and no soft tissue remains. Mummification at the FARF is characterized by a uniform beige or brown color, with a very thin, paper-like skin remaining over the skeleton. Complete mummification occurs between 11 and 106 days after placement (240.9-1697.7 ADD). Skeletonization, the last decomposition stage studied for this project, occurs when the complete skeleton is visible. Skeletonization presented in only 30% of the donations initially analyzed, and of those individuals, only half-skeletonization occurred. When data from the 2012 donations are applied to the Universal PMI formula introduced by Vass, a significant difference is found in the stages of early decomposition (x^2=251.49), late decomposition (x^2=291.85), and mummification (x^2=216.1) between the observed PMI and the PMI expected from Vass' formula.

When these data are used in collaboration with decomposition data acquired from different regions, a more accurate PMI can be established. Initial results vary greatly from other regions previously studied, and this shows the necessity of region-specific PMIs. This project builds upon previously collected data and adds new data that are part of an ongoing standardized decomposition database that includes photos and notes that will be beneficial for researchers interested in standardizing the visual assessment of decomposition.

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Decomposition, Postmortem Interval (PMI), Forensic Anthropology Research Facility (FARF)

H88 Craniometric Assessment of Modern 20th-Century Black, White, and "Colored" South Africans

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After attending this presentation, attendees will obtain knowledge of human variation among modern Black, White, and "Colored" South Africans and will understand the statistical framework used to describe similarities and differences among these groups.

This presentation will impact the forensic science community in contributing to the knowledge of human variation in modern South Africans as well as the impact of forced segregation on cranial plasticity in different ancestral populations occupying the same geographic space.

While South Africa is not unique in its rate of violent crime or in its large number of unidentified persons, this poly-linguistic society with over 49 million people is an ideal country in which to evaluate human variation. Within the last 500 years, various population groups, such as Dutch, French, Malaysian, and Indian, have migrated to South Africa. Social behavior and 20th-century segregation laws affected gene flow among indigenous and migrated groups and contributed to distinct social/political designations which include Black, White, "Colored," and Indian South Africans. Approximately 80% of the population identifies themselves as Black, 9% "Colored," 8% White, and 3% Indian. "Colored" refers to a social group primarily from the Western Cape who are descendants of slaves brought from Indonesia, India, Malaysia, and Asia and who mixed with Europeans and the indigenous Khoi and San.[1]

The purpose of this study was to use craniometrics and Discriminant Function Analysis (DFA) to evaluate ancestral variation and sexual dimorphism among White, Black, and "Colored" groups as a means to explain current variation and to more accurately identify unidentified remains as to social group.

A total of 351 crania of Black (49 F, 110 M); White (45 F, 64 M); and "Colored" (29 F, 54 M) groups were used from the Pretoria Bone, Raymond A. Dart, and Kirsten skeletal collections. One-hundred and seven standard landmarks were digitized to generate various linear measures, fractures, angles, and subtenses.[2] Discriminant function analysis was employed and South African groups were tested against themselves to test classification accuracies. All accuracies were cross-validated.

In a three-way DFA for ancestry using 11 stepwise-selected variables, 82% classified correctly using cross-validation, indicating significant differences among all groups. Black South Africans classified 80% correctly, "Coloreds" classified 84% correctly, and Whites classified 83% correctly. In a six-way DFA for sex and ancestry using ten stepwise-selected variables, 63% classified correctly on the whole. Black females classified 76% correctly, Black males classified 74% correctly, "Colored" females classified 24% correctly, "Colored" males classified 41% correctly, White females classified 69% correctly, and White males classified 66% correctly.

Each ancestral group demonstrated comparable correct classification accuracies when tested for ancestry alone. Of the three groups, Black and White groups were least likely to misclassify as each other. For ancestry and sex, "Colored" groups demonstrate considerably lower classification accuracies than either Black or White groups. "Colored" males misclassify into Black or White groups, whereas "Colored" females misclassify more often as Black females than themselves and only twice as White females. The abovementioned results corroborate with nasal aperture research on South Africans which concluded that while Black and White groups were distinctly different, no clear discrimination could be made between Black and "Colored" groups.[3] Low classification accuracies for sex and ancestry were also observed and may be attributed to low sexual dimorphism among "Colored" and Black groups, despite strong separation of the groups in a three-way DFA.[3]

Diverse cultural/sociopolitical histories are reflected in variation among these modern social groups. Few studies have examined morphological variation within "Colored" groups in comparison to White and Black South Africans. While Black and White South Africans show roughly the same level of homogeneity, "Coloreds" are much more heterogeneous. Current variation in South Africa needs to be addressed to assist forensic anthropologists in accurately estimating ancestry from unknown remains.

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Ancestry Estimation, DFA, Sexual Dimorphism

H89 The Chaotic Numerology of Anthropometry: A Proposal for a Univocal Numeric Codification of Bone Measurements

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After attending this presentation, attendees will value the state-of-the-art anthropometrical measurement's classification and develop a better understanding of a new code that could be adopted by international scientists.

This presentation will impact the forensic science community by illustrating a new univocal and versatile three-number code for cranial and skeletal measurements.

Osteometrics have very important roles in forensic anthropology because they allow for the objective quantification of morphological characteristics when developing the biological profile of unknown skeletons, rather than relying purely on qualitative descriptions that are often subjective.

Various measurement coding systems have been developed in Europe and the United States; some of the most popular include those developed by Martin-Saller, Howells, and by Buikstra and Ubelaker.[1-3] As a scientific community that is becoming ever more global and

international, a nonshared language can create impasses and lead to miscommunications between scientists. Similar problems have been faced by odontologists that are involved in mass fatality and international scenarios. The odontological community has addressed this problem with the FDI World Dental Federation notation (i.e., the ISO 3950 notation), where quadrants are numbered from 1 to 4 for adult dentition and from 5 to 8 for deciduous dentition. The numbers proceed clockwise from the upper right quadrant to the lower right and teeth are numbered from the midline to the posterior.

In order to develop a new shared codification model, some imperfections in the main coding systems must be overcome. For instance, one issue with the Martin-Saller system is that all measurements are divided in chapters corresponding to single bones and are numbered with an arithmetic progression. This system can be ambiguous because the numbers are not univocal: for example the number "1" indicates the maximum length of the skull, of the femur, and of all the other long bones. The Howells coding system identifies measurements by an abbreviation of its description, which is problematic when a measurement has a very long or complex name, or if new measurements are acquired. The system developed by Buikstra and Ubelaker is problematic because codes are sequentially assigned from the skull to the calcaneus, so new measurements cannot be easily introduced into the sequence without creating confusion.

A coding system based on a three-number codification, where numbers are delineated by periods is proposes. The first number will indicate the anatomical area of which the measurement is referred:

- 1 – Cranium
- 2 – Upper Limb
- 3 – Lower Limb
- 4 – Spine
- 5 – Thoracic Girdle
- 6 – Pelvic Girdle

The second number will indicate the single bone or the topographic region in the cranium:

- Neurocranium, 1.2 Facial Skull, 1.3 Orbital Skeleton, 1.4 Nasal Region, 1.5 Maxillary Area, 1.6 Mandible
- Humerus, 2.2 Ulna, 2.3 Radius
- Femur, 3.2 Tibia, 3.3 Fibula, 3.4 Patella
- Vertebrae, 4.2 Atlantoaxial Joint, 4.3 Sacrum
- Scapula, 5.2 Clavicle, 5.3 Sternum
- Innominate, 6.2 Pelvis

The first two numbers in the code can be used to rapidly identify the anatomical area, where the bone is in the body, and in which bone the anthropometrical data is recorded; the third and last number is an arithmetic progression that allows operators to continually introduce new measurements without scrambling the entire series. A selection of the most representative measurements are selected and presented on the web site www.restiumani.it.

This presentation outlines a novel anthropometric coding system. The proposed coding system is an integral part of the "Forensic Protocol for Anthropometric Measurement of Human Skeletal Remains" developed at the University of Tor Vergata. The coding system and the protocol have been successfully utilized on a variety of historical and forensic Italian cases in a five-year research project at the University of Florence and are used by various Italian universities and by forensic investigators for the Italian State Prosecutor's Office.

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Anthropometry, Standards, Codification

H90 Defining the Peri-Mortem to Postmortem Transition: Macroscopic and Microscopic Changes in Subadult Bone Undergoing Trauma

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The goal of this presentation is to more clearly and accurately define the peri-mortem period in forensic anthropology by documenting changes in traumatized subadult bone across the peri-mortem to postmortem transition.

This presentation will impact the forensic science community by providing a better understanding of the peri-mortem /postmortem transition and enable practitioners to more clearly distinguish between peri-mortem versus postmortem pediatric trauma.

Scientific Working Group for Forensic Anthropology (SWGANTH) guidelines for trauma analysis[1] have called attention to the need for great caution when using the term "peri-mortem" due not only to different definitions of the term across soft and hard tissue, but to a paucity of understanding of the timing and process involved in fresh bone becoming "dry."[1] One approach has been to define this transition in terms of characteristic signatures related to biomechanical properties of "fresh" versus "dry" bone breakage. Using this approach, retention of classic macroscopic peri-mortem Blunt Force Trauma (BFT) signatures has been observed well into the postmortem period (five months); however, a discrete, standardized definition and timing of this transition has been elusive.[2]

The current study documents microscopic and macroscopic changes in subadult bone undergoing BFT across the peri-mortem to early postmortem transition (approximately seven weeks) (peri-mortem is defined in this study as at or very near the approximate time of death). Twenty (unprocessed) stillborn pigs (*Sus scrofa*), frozen at death, were used in the study. After thawing, two pigs underwent immediate trauma induction delivered by means of a standardized drop-force mechanism. Pigs were placed on a hard substrate and impacted by a 1,109g concrete cylinder on both their left and right sides. Right side impacts (three per pig—focused on the lateral cranium, lateral shoulder, and ribs) used the same standardized drop mechanism through a stabilized 50cm-long PVC pipe. Similarly, three impacts on the same areas (cranium, shoulder, ribs) on the left side were dropped through a 108cm-long PVC pipe. Pigs were radiographed after trauma induction, then underwent maceration. The remaining 18 pigs, immediately upon thawing 24 hours, were placed in outdoor and indoor surface environments in the summer season to decay. All were placed in cages lined with ¼ in, fine wire mesh to allow access to insects, but limit larger scavenging and allow natural decomposition to occur. At regular intervals over the seven-week period (Days 5, 10, 20, 35, and 50), three pigs from each environment (two outdoor, one indoor) underwent identical trauma induction, radiography, and processing. The final two pigs (one outdoor, one indoor) remained in their environment for the entire 50 days, undergoing no trauma.

Macroscopic and microscopic (using a Keyence VHX-1000 Digital Light Microscope with 5 – 50x and 20 – 200x lenses) comparisons were made across the Day 0 through Day 50 bone samples. Standardized variables examined included:

- Fracture (Fx) type (linear, hinge, diastatic, depressed, comminuted, stellate), frequency, completeness;
- Fx morphology (including fx outline, fx edge shape);
- Fx metrics (e.g., distance between incomplete fx edges; length of radiating fx lines);
- Presence/absence of hinging, radiating and/or concentric fx lines, inbending/outbending, displacement, curling/uplifting; degree of refit of fx edges.

Results indicate a change from many classic peri-mortem fracture signatures to postmortem ones early in the postmortem period (by Day 5). This includes an increase in overall fragmentation as indicated by fracture frequency (particularly those of ribs) between 0 and 5 days

postmortem, a pattern continuing until Day 50. Color differentiation between fractured and non-fractured surface edges is visible by Day 5. Decreases in sharp-edged denticulate fx edges and increases in jagged, irregular, and frayed fx edges are observed by Day 10. There is a concomitant decrease in the number of possible refits across this period. An illustrated timeline for microscopic and macroscopic bony changes across this peri-mortem/postmortem transition is provided. It is recommended that a standardized fracture terminology in the description of peri-mortem trauma and postmortem breakage be adopted, through quantifiable microscopic and macroscopic comparisons. These results will aid in the differentiation of peri-mortem versus postmortem pediatric fractures and further clarify both the nature and the timing of the peri-mortem period.

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Peri-Mortem, Postmortem, Pediatric Trauma

H91 Quantification of Color Changes in Human Decomposition Using Image Processing Software

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After attending this presentation, attendees will understand how image processing software can quantify color changes in human decomposition. This presentation will show how the software can be used to construct a chromaticity diagram based on the RGB and CIELAB values generated from a set of images taken for a study on postmortem interval estimation by a total body score and accumulated degree-days method.

This presentation will impact the forensic science community by exploring the relationship between temporal changes in human decomposition and changes in chromaticity diagrams. It will also explain how RGB and CIELAB values taken from digital images might be used to validate current postmortem interval estimation.

Scientists can use a spectrophotometer to measure quantitatively the transmission or reflection properties of a material as a function of wavelength.[1] The spectral wavelengths are converted into three values, known as CIELAB tristimulus values, which numerically represent the sensitivity curves of three linear light detectors. Then, the values are used to calculate the two parameters humans use to process color: chromaticity, a quantitative property that is a combination of luminance, hue, and saturation; and brightness, a purely qualitative property. The parameters are plotted as x and y coordinates on what is known as a CIE 1931 color space chromaticity diagram.

Dermatologists have used spectrophotometry to monitor skin lesions and color changes in patients' skin.[2] While spectrophotometry is an accurate method of quantifying color change, it requires direct contact with the material being analyzed. Not all lesions or conditions allow for direct contact with this instrument of analysis, nor is it suitable for all types of investigation. Ophthalmologists, for example, cannot use a spectrophotometer inside the eye to diagnose diseases such as macular degeneration.[3] They instead use a method known as digital colorimetry. Image processing software analyzes an image or any specific region of an image and generates a set of values known as RGB values. These RGB values are another set of color additive values and can be converted into the same tristimulus values that can be plotted on a chromaticity diagram.

The stages of human decomposition can be quantitatively classified using a system like the Total Body Score method.[4] While the scoring system is a quantitative measure, this system and others like it rely on color as a criterion for classification. Large changes in color are easy to detect, but reporting the overall color of a limb or torso at only one point in time might be difficult. Investigators need a quantitative way of evaluating color, or at least a quantitative method of validating their estimation.

Spectrophotometry would be an accurate technique in analyzing color in human decomposition, but it can be problematic. The area of analysis is restricted by the need for direct contact with the body. Forensic scientists might need to analyze the colors on a large part of the body or even on the body as a whole before they classify the stage of decomposition. Digital colorimetry is a comparable alternative. It uses the digital images of the bodies at the crime scene or recovery scene, and immage processingsoftware can analyze specific or larger regions of the images.

Six digital images representing all stages of human decomposition will be selected from a larger group of images taken for a previous study.[5] ImageJ software will be used to select a region in each photo and will generate the respective RGB values; the images analyzed will not be corrected or altered. The RGB values will be converted into chromaticity coordinates and will then be plotted on a CIE 1931 color space chromaticity diagram so that a trend in the values can be evaluated.[6] This process will be repeated on the images after correcting them for brightness and contrast.

Preliminary results show RGB values generated from both uncorrected and corrected images can be converted into CIELAB values, which can then be plotted on a chromaticity diagram. The red, green, and blue color channels will be plotted against the time progression to establish how strong a relationship exists. Differences in the sets of values will expose the necessity of image correction. Patterns in the plotted points will reveal how digital colorimetry tracks color changes, and if it can validate postmortem interval estimation.

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Human Decomposition, Chromaticity Diagram, Digital Colorometry

H92 Wearing Down Old Perspectives: The Prevalence of Severe Dental Attrition in Modern Individuals

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After attending this presentation, attendees will have a better understanding of the frequency of severe dental attrition in modern individuals, as well of the possible causes of this condition.

This presentation will impact the forensic science community by highlighting the presence of severe tooth wear in forensic cases, which is typically observed only in archaeological contexts. Additionally, the

researchers explore how this degree of wear can affect the development of the biological profile and the process of identification.

In the analysis of skeletal remains in the United States, the presence of severe dental attrition is usually an indicator of ancestry (i.e., Native American) and can serve as a deciding factor in determining medicolegal significance. However, attrition occurs in all populations for a variety of reasons. For example, 121 contemporary individuals from the William M. Bass Donated Skeletal Collection were examined for severity of tooth wear in the posterior (premolar and molar) and anterior (incisor and canine) teeth. Large areas of exposed dentin, as indicated by a wear score of five or higher according to Smith, were observed in the posterior dentition of 14% of the sample, and in the anterior dentition of 12% of the sample.[1] While a relatively small percentage of the sample exhibited a severe wear score, none of the individuals fit the typical demographic or contextual profile for this degree of wear (e.g., prehistoric).

Possible causes for severe attrition in contemporary individuals were explored through the examination of the skull of a male in his early 40s exhibiting a degree of dental attrition akin to prehistoric populations. Although this condition typically rules out medicolegal significance in the United States, three key lines of evidence indicated that he was a modern individual. First, the remains were in an advanced state of decomposition upon discovery. Second, the individual displayed evidence of antemortem modern cranial surgery. Third, several modern dental restorations were present. Without these indicators, time since death for this individual may have been determined to be outside the realm of medicolegal significance; as such, these remains may never have been identified.

A number of hypotheses for the presence of this extreme attrition were examined through a review of the literature and a cross-disciplinary discussion with dental professionals. Hypotheses considered included wear resulting from this individual's particular occlusal alignment, wear related to subsistence strategies or other nonmasticatory use, and pathological bruxism secondary to neurotrauma. The investigation suggested that fewer and fewer researchers consider occlusal alignment to be a prominent factor in bruxism.[2] Although the individual exhibits edge-to-edge occlusion, the researchers surmised that this morphological factor did not play a significant role in the progression of attrition. Further review found that the individual exhibits a wear pattern unlike any previously documented population-specific wear.[3] Thus, cultural influence is an unlikely factor. Finally, literature describing similar wear in patients with neuromuscular disorders suggested that severe wear can occur over a relatively short time period due to constant diurnal and nocturnal bruxism.[4] Research also showed that disturbances in the central neurotransmitter system of the brain, specifically the basal ganglia, are directly linked to severe bruxism.[2] Therefore, the researchers conclude that this individual's advanced state of attrition was likely associated with the antemortem cranial trauma and resulting neuromuscular complications.

The frequency of tooth wear observed in the Bass Collection, as well as the presence of severe attrition in a fairly young individual, illustrated the necessity to consider the impact of severe attrition on the forensic identification process. Specifically, consideration of the full range of possible factors associated with tooth wear is essential in determining medicolegal significance. Additionally, the fact that this degree of wear occurs secondary to neurotrauma over a relatively short period of time is important in instances where tooth wear may be used for aging, as it would result in a significant overestimation of age. Forensic anthropologists should be aware that encountering similar modern cases of severe attrition may be more common than previously thought. Special attention to causal factors is required in these cases to determine the possible effects on the development of the biological profile.

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Dental Attrition, Bruxism, Neurotrauma

H93 Biomechanical Evaluation of Frangible Skull Surrogates to Blunt Ballistic Temporo-Parietal Head Impact

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After attending this presentation, attendees will obtain additional knowledge on the biomechanical testing of trauma-indicating skull surrogates for use in forensic investigation.

This presentation will impact the forensic science community and aid attendees by providing additional morphological fracture data from laboratory-based testing of Postmortem Human Subjects (PMHS) exposed to blunt impacts in additional to a biomechanical comparison between the PMHS and frangible skull surrogates.

Various breakable or frangible "trauma indicating" head models have been proposed over the years by the automotive safety community.[1,2] Effort was shifted to the improvement and development of nondestructible technologies with frangible models never making it to a production level. The need for a frangible head model has since resurfaced for forensic applications. Forensic applications of injury biomechanics is a unique and emerging field.

The "skin-skull-brain" model was developed by Thali et al. to reproduce fracture patterns seen in forensic cases due to blunt and ballistic traumata.[3,4] Known cases of blunt and ballistic traumata were used as validation of the model's fracture characteristics. While good agreement was found between fracture patterns, no biomechanical considerations such as biomechanical response (force and deformation characteristics) or fracture tolerance were addressed in the study.

The goal of the current research was to evaluate the biomechanical response and fracture tolerance of a frangible skull model to blunt and ballistic traumatic conditions. Biomechanical response, fracture tolerance, and resulting fracture patterns were compared to those produced in PMHS exposed to the same traumatic conditions. Two types of frangible surrogates were evaluated. Three material types (10% gelatin solution, lead shot surrounded by Styrofoam, and water) were used to represent intracranial contents to evaluate the effect of interior boundary condition. A leather chamois was used as a skin covering over the impact site. A 103 gram, 1.5 inch-diameter impactor was launched at 20 m/s from a custom air cannon at the surrogate/PMHS which was suspended via a lightweight cable. An accelerometer was embedded in the impactor to measure impact force via Newton's Second Law. High speed digital video was captured at 10,000 frames per second with two Kodak HG100k cameras mounted orthogonally to the specimen. PMHS specimens were tested with soft tissue intact for the first impact. Following the first impact, a second impact was performed following defleshing of the specimen to obtain forced-deformation response under both conditions for comparison. Force-deformation plots were generated from the PMHS tests for comparison to the frangible surrogate responses. Fractures in PMHS were photographed for morphological comparison to the surrogate tests.

The sphere designs investigated in the current study demonstrated an increased tolerance to fracture compared to PMHS. The fracture pattern created in the one fractured sphere did not compare well with the PMHS fracture patterns, but may be an artifact of the seam created by joining the two-piece skull model. The effect of internal boundary condition was evaluated by assessing three different brain substitutes. The 10% gelatin solution provided the maximum resistance to local deformation while the lead shot surrounded by Styrofoam provided the least resistance. The water-filled sphere resulted in the most biofidelic

force-deformation characteristics indicating it may be the more ideal internal boundary condition. Additional testing is necessary before statistical conclusions can be reached.

These results indicate material properties (polyester versus polyurethane) and structural composition are important factors that should be evaluated for the current frangible skull surrogate design. In addition to modifications to the structure of the model, consideration is needed for a biofidelic soft tissue covering.

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Bone Trauma, Skin-Skull-Brain, Biomechanics

H94 Interpreting the Injury Mechanisms of Blunt Force Trauma From Butterfly Fracture Patterns

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After attending this presentation, attendees will have a better understanding of: (1) which factors affect butterfly fracture formation; and, (2) how to interpret the direction of applied force from butterfly fracture patterns. The main goal of this research was to challenge previous assumptions made about the interpretation of blunt force injury mechanisms from butterfly fractures and to provide the forensic community with rigorously tested results on how and whether the direction of force can be accurately determined from butterfly fracture analysis.

This presentation will impact the forensic science community by demonstrating that the side and orientation of butterfly fracture fragments can be inconsistent and, therefore, inaccurate when used as the sole criterion for determining the direction of applied force; however, this study has also found that by analyzing the pattern of the fracture in its entirety for both complete and incomplete fractures, a specific blunt force fracture pattern can be observed and used to more accurately determine the direction of force.

The majority of current forensic literature suggests that the direction of applied force can be determined by analyzing the orientation of butterfly fracture patterns. More specifically, the side of the bone from which a butterfly-shaped fragment originates is thought to directly correspond to the direction of applied force.[1] The use of this method was recently cautioned by Thomas and Simmons after analyzing the controlled fractures of 94 sheep femora.[2] This study reported the formation of butterfly fractures to be highly inconsistent, observing butterfly fragment formation on both the impact and nonimpact side of bones. Another recent study by Fenton et al. analyzed the controlled fracturing of 15 dry human long bones.[3] This study reported that the direction of force could be determined in 14 out of 15 cases by analyzing the entire fracture pattern, including both gross and incomplete fracture lines, instead of focusing merely on the identification and orientation of butterfly fragments. These studies indicate that further controlled research is needed to corroborate, refute, or simply clarify the accurate interpretation of the direction of applied force from blunt force fracture pattern analysis.

In this study, 105 *Ovis aries* femora were broken using a customized pendulum impact apparatus made of a large wooden frame with a weighted base, a rigid metal pendulum, blunt anvil, and an adjustable bone stand with metal cup stabilizers. The bones were oriented in an upright position with the anterior mid-shaft surface facing the impact hammer. The bones were secured in place by adjustable metal cups that applied compressive forces in a fashion mimicking the intrinsic forces experienced by weight bearing.[4] The initiation and propagation of each fracture was captured with a high-speed camera at 1,000 frames per second.

According to the biomechanics of fracture production, the biomechanical response of a bone to applied extrinsic forces is highly dependent on its specific geometry; therefore, the following measurements and observations were recorded for statistical analysis: length; mid-shaft circumference; cortical thickness; moment of inertia; fracture classification; presence of butterfly fragment; fragment angle; fragment side; and overall fracture pattern and propagation.[4] In addition to this experiment's sample, the 94 sheep femora previously broken by Thomas and Simmons were re-analyzed with the intent of including both gross and incomplete fracture lines in the analysis of determining the direction of applied force.[2] Including this sample also allowed for a comparison between two different injury mechanisms; more specifically, between the fractures seen in bones held upright with compressive forces and bones held against a substrate.

Preliminary results suggest the wedge-shaped fragmentation traditionally described as a butterfly fracture pattern does, as reported by Thomas and Simmons, occur on both the impact and nonimpact side of the bone.[2] However, refocusing the analysis on the entire fracture pattern, as suggested by Fenton, reveals a specific pattern of gross and incomplete fractures in 76% of the sample.[3] Based on these observations, a new overall fracture pattern was defined. Subsequently, it was found that the direction of applied force could be determined accurately in 100% of specimens displaying a partial or complete variation of the identified pattern. At this time, no comparison has been made between the bones held upright with compression and the bones held against a substrate; however, this comparison will be addressed in the presentation.

In summary, this study demonstrates that the side and orientation of butterfly fracture fragments is indeed inconsistent and, therefore, inaccurate when used as the sole criterion for determining the direction of applied force. This study showed that through analyzing the pattern of the fracture in its entirety for both complete and incomplete fractures, a specific blunt force fracture pattern is observed and can be used to accurately determine the direction of force.

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Butterfly Fracture, Blunt Force Trauma, Injury Mechanisms

H95 Reported and Observed Healed Fractures in a Modern Skeletal Collection

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After attending this presentation, attendees will be informed about reported medical history data of a modern skeletal collection and the reliability of information regarding reported fractures.

This presentation will impact the forensic science community by increasing the knowledge of the reported data on the individuals in the W.M. Bass Donated Skeletal Collection, thus guiding future research

questions related to the medical history. It will also present healed fracture patterns observed in the collection and discuss how reliable the family-reported data is and whether it is applicable for identification purposes.

Forensic anthropology uses modern skeletal collections to create and test its methods. These collections usually have known demographic and other information for each individual. The information can be from different sources: self- or family-reported or measured. It is important to know the reliability of the reported information. For example, there are studies on the reliability of reported stature and weight information in the literature.

This presentation concentrates on the W.M. Bass Donated Skeletal Collection and the reported medical history of the individuals included in the collection, specifically healed fractures. This body donation program has a questionnaire for pre-donors and family members to complete. The questionnaire collects data on demographics, education, residence history, dental history, and medical history. The medical history section includes check boxes to document surgeries, fractures, amputations, prosthetics, diabetes, and cancer. Also, blank spaces are available for more detailed descriptions.

The focus of this study was to identify differences between the reported and observed fractures and to investigate possible sex dependence. The study population consisted of 160 White males and females. The age range of the population was 34 to 93 years, and females (average age 65 years) were slightly older than males (average age 62 years).

More self-donors reported fractures than family-donors. The number of individuals with reported fractures in males and females was nearly equal. Self-donors and family-donors had the same number of individuals with observed fractures. More females were observed to have at least one fracture than males.

An arm or spine fracture was the most common injury reported in females; an arm or leg fracture was the most common injury reported in males. Arm and leg fractures were reported more often by family-donors and arm and spine fractures more often by self-donors. Ribs and spine were fractured most frequently within both sexes.

Self-donor males reported the highest occurrence of fractures; 75% of the reported fractures were observed on the skeletal remains. In comparison, 77% of the fractures reported by self-donor females were observed; whereas, less than 60% of fractures reported by family donors (for both males and females) were observed. In general, the most under-reported fractures were rib fractures. Arm fractures were often reported and observed, while leg fractures were often reported but not observed.

The study of self- and family-reported fracture data has several limitations. Several individuals note a history of fracture(s), but do not provide specifics such as location. More often a leg, ankle, wrist, or arm is mentioned, not the specific bone or side. Other possible reasons for mismatch between the observed and reported fractures are missing or fragmentary elements, well-healed old fractures, fractures that occur after the self-donor questionnaire is completed, unawareness of the fracture, or forgotten fractures.

In conclusion, based on these data, the reliability of the reported information on fractures depends on the reporter (self-donor or family member) as well as the type of fracture. Other factors to be taken into consideration are how old the fracture is and how long ago the injury was reported.

Antemortem Fracture, Reported Data, Identification

H96 The Use of the Endocranial Base in the Estimation of Ancestry

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The goal of this presentation is to inform attendees about an evaluation of selected internal (endocranial) base landmarks performed on crania from the Hamann-Todd Human Skeletal Collection, and to test the merits of inclusion of endocranial base landmarks in forensic analysis.

This presentation will impact the forensic science community by demonstrating the utility of endocranial base landmark analysis in estimation of ancestry on a large sample of known remains.

Ancestry assessment is an integral part of the analysis of human remains in forensic studies. The cranium is among the most useful parts of the skeleton for estimation of sex and ancestry. In metric analyses, ancestry assessment has traditionally relied on linear distances between landmarks on the external (ectocranial) surface of the cranium.[1-3] In particular, in forensic settings a current analytical tool is the multivariate group comparison of these interlandmark distances using the software FORDISC 3.[4]

The endocranium, in particular the endocranial base, is easily accessed after an autopsy cut routinely performed in forensic settings, and contains several consistent, easily identifiable anatomical landmarks. The endocranium is also very durable, and is often well preserved in fragmentary remains. Since similar developmental pressures affect both the internal and external surfaces of the cranium, it is likely that, like ectocranial landmarks, endocranial landmarks can be useful to discriminate different sex and ancestry groups.

Studies assessing the utility of endocranial landmarks are scarce in the literature. Cameron devised angles about the pituitary point on sagittal sections of crania from the Hamann-Todd Human Skeletal Collection to evaluate cranial flexion, which was revisited by May and Sheffer and Lieberman et al. in extant primates and modern humans.[5-7] Bruner and Ripani, in a forensic application of endocranial landmarks, evaluated the utility of 19 endocranial base points for sex estimation, finding that all detected sex differences were found to be primarily related to allometry, they did not evaluate differences in ancestry.[8]

In this study, the utility of a set of endocranial landmarks is assessed for ancestry determination using landmarks proposed by Bruner and Ripani, as well as an additional set of landmarks proposed by the author. A 3D digitizer was used to digitize landmarks on 200 crania from the Hamann-Todd Human Skeletal Collection. Both interlandmark distances and Procrustes coordinates were analyzed through discriminant function analysis using FORDISC 3 and a geometric morphometrics software. Sex differences were attributed to size, so they were detected in interlandmark distance measurements but not in Procrustes coordinates, which eliminate size differences, focusing solely on shape. When the sexes were pooled, shape differences between ancestral groups were found. Similar to traditional ectocranial landmarks, endocranial base landmarks showed significant differences between African American and European American crania, with African Americans displaying significantly longer and narrower cranial bases. Discriminant function analysis using FORDISC 3 classified ancestral groups with a cross-validated accuracy of 77.3%.

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Endocranial, Base, Ancestry

H97 3D-CT Imaging and Global Crime Scene Reconstruction in Forensic Ballistics—Two Case Reports

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After attending this presentation, attendees will have an enhanced understanding of 3D reconstruction of external and internal bullet tracks in case of gunshot wounds.

This presentation will impact the forensic science community by highlighting the value of virtual tridimensional reconstruction of both crime scene and Computed Tomography (CT) images to help in determining the course of events in forensic ballistics.

Postmortem investigations are increasingly assisted by Multislice Computed Tomography (MSCT). Over the past twenty years, these examinations, often termed *virtual autopsy* or *virtopsy*, have become more available to forensic pathologists. The aim of the presented study was to examine ballistic injuries using advanced radiological techniques and comparing the results with the autopsy findings. In cases of ballistic injuries, CT scanning and Three-Dimension (3D) reconstructions provide an accurate description of the bullet location, bone fractures, and, more interestingly, a clear visual of the intracorporeal trajectory (wound tracks). These forensic medical examinations are compared with tridimensional bullet trajectory reconstructions made by forensic ballistics experts.

The implementation of tridimensional methods and the results of the global crime scene reconstruction is shown through two case reports.

Case 1: A 33-year-old woman was shot in the shoulder by a twelve-gauge smooth-bore firearm with slug ammunition (Brenneke). The 3D-MSCT revealed the presence of numerous bullet and bone fragments and a trajectory of anterior-posterior, right-to-left. Two wound tracks were identified: the primary track that coursed from the entrance to the exit wounds; and a secondary track that terminated with small bullet fragments. The examination allowed the isolation of two possible scenarios for the external bullet trajectory. Both were modeled with the appropriate software. The confirmation of internal and external bullet trajectories confirmed the defendant version and showed the incompatibility of the victim's initial statements with the forensic findings.

Case 2: A 32-year-old man was found in a burned car with burn injuries corresponding to a Crow-Glassman level 3. A bullet (full metal jacket with lead core and brass jacket, .25 ACP) was found in the skull. The 3D-MSCT revealed a linear cranio-encephalic trajectory, strictly horizontal and right-to-left. The 3D crime scene reconstruction compared with forensic medical examination presented the best scenario, which was a shot from outside the car.

These case reports highlight the usefulness of CT imaging techniques for the reconstruction of ballistic trajectories. Virtual autopsy can be used to reliably reconstruct gunshot wound tracks, which are generally linear tissue defects associated with gas, bone, and metallic fragments. Postmortem CT imaging is, therefore, an important and useful complement to the forensic autopsy. Work collaborations between police forensic experts and forensic medicine institutes allow the incorporation of medical examination in a global crime scene reconstruction. CT imaging reconstruction is an interest in forensic science, and it's also a clear visual communication tool between experts and the court.

Forensic Ballistics, 3D MSCT Reconstruction, Bullet Trajectory

H98 The Analysis of Insect Succession Rate and Pattern of Decomposition in Charred, Clothed Remains

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After attending this presentation, attendees will gain an understanding of the effects of accelerants on the rate and pattern of decomposition.

This presentation will impact the forensic science community by providing important information regarding Postmortem Interval (PMI) estimations for forensic investigations and a broader understanding of factors that affect decomposition.

Clandestine fires are common in criminal settings where fire is used to destroy evidence which may link the perpetrator to the crime or to obscure the victim's identity.[1] The offender may cover the body in accelerant before ignition, while underestimating the amount of fuel and heat required, thus producing only partially charred remains.[2,3] Offenders use easily accessible accelerants, with petrol employed in most cases (50.4%) and paraffin in fewer (28%).[4] Due to the nature of clandestine fires, the victim's body is often not recovered immediately; therefore, the PMI estimation becomes forensically relevant because it allows an approximation of the date of the incident and may aid the identification process.

There is a paucity of literature regarding the decomposition of charred remains and techniques for the approximation of the PMI. Given the awareness that fire is employed in homicides to destroy human remains and the frequency of lengthy delay in discovery, the presence of this gap in the literature is surprising. Gruenthal *et al*. (2012) represent the single attempt in PMI approximations in remains charred to Crow-Glassman System (CGS) Levels 1 and 2.[5] This research provides an approach to the estimation of the PMI in accelerant-charred remains and aims to determine whether or not the use of these accelerants in burning the remains had a significant effect on insect succession (Coleoptera and Diptera), pattern, and rate of decomposition.

At the beginning of the experiment, 30 domestic pig carcasses (*Sus scrofa*) were transported to the Taphonomic Research in Anthropology Centre for Experimental Studies (TRACES), University of Central Lancashire. The pigs were separated into three groups and were washed and weighed, and thermocouples with data loggers were inserted rectally before dressing the pigs in T-shirts and shorts. The pigs were then placed in their field locations five meters apart, and treatment carcasses were covered with 2.5 liters of their respective accelerant before ignition, within minutes of application. The fires burned out naturally, and the extent of thermal destruction was scored using the Crow-Glassmann System (1996).[5] Each pig was covered in a wire mesh cage to prevent vertebrate scavengers from accessing the carcasses.

Data collection intervals were approximately every 30 – 40 Accumulated Degree Days (ADD). During each data collection, the treatment carcasses were scored using both the Gruenthal et al. (2012) and the Megyesi et al. (2005) scoring systems while the control carcasses were scored using only Megyesi et al. (2005)[6,7]. Data loggers were used to record ambient and internal temperatures (°C) at 6-hr intervals.

Data analyses were carried out using statistical software, R (V 2.9.3). A comparison of the two scoring systems for the accelerant-charred remains was carried out using a mixed effects model, and indicated both scoring systems can be applied with equal success for decomposition in accelerant-charred remains (r^2=0.89).[6,7] Rate of decomposition was analyzed using linear regression Analysis of Covariance (ANCOVA) with Total Body Score (TBS) as the response variable, and treatment and ADD as explanatory variables.

After 540 ADD, results showed the control group decomposed significantly faster than the burned groups ($p \leq 0.001$), while petrol decomposed significantly faster than the paraffin ($p \leq 0.001$).

Pattern of decomposition was scored separately for each of the three regions. The head and neck region was significantly different between the control and the two experimental groups ($p \leq 0.001$); there were no significant differences among any of the groups in the torso and limb regions.

The preliminary results suggest both scoring systems can be used for assessment of accelerant-charred carcasses. Accelerant-burned bodies decomposed significantly more slowly than unburned bodies. In particular, the head region of the treatment groups decomposed significantly more slowly than that of the controls. It is hypothesized that the presence of accelerants masks the volatile odors produced during early decomposition, thus delaying blowfly oviposition, and postponing the consumption of the carcass by maggots.

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Burning, Accelerants, Decomposition

H99 Quantifying Heat Exposure of Osseous Material Utilizing Novel FTIR Peak Ratios

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After attending this presentation, attendees will have gained an understanding of how indices calculated from Fourier Transform Infrared Spectroscopy-Attenuated Total Reflectance (FTIR-ATR) absorption peaks can give vital information regarding the burn temperature and exposure time of bone that has been subjected to fire.

This presentation will impact the forensic science community by reforming traditional methods of predicting heat exposure of bone and significantly improving the rate of accurate temperature prediction in experimental burnings from 19% using the traditional method to 82% when incorporating the new ratios.

Bone can reveal insightful information about an individual's life and circumstances of death. Professionals of various disciplines have an interest in understanding the changes bone undergoes when subjected to fire, be they archaeologists investigating ancient burial practices or forensic personnel confronted with the reconstruction of a scene of accident or crime.

Traditionally, only the so-called Crystallinity Index (CI), which is defined by the splitting of the two absorption peaks at 605 and 565cm^{-1} when using spectroscopy as a mean of analysis, has been used to gain an understanding of the heat exposure bone has undergone. The higher the exposure temperatures, the more ordered the bone structure becomes, exhibiting larger hydroxyl apatite crystals; however, this trend is reversed at temperatures above 800°C, due to the complete loss of all organic components and a shift in the elemental ratios. The consideration of the entire FTIR spectrum, therefore, lends itself for a better understanding of heat-induced changes to the bone matrix.

The present study investigated the use of new absorption peaks for the determination of burning temperatures and duration. Research was carried out using defleshed rib bones of domestic sheep (*Ovis aries*) which were experimentally burned in a furnace for 45 min at temperatures between 50°C and 1100°C in 50°C increments. For each temperature, two bones were burned, each of which was sampled nine times, resulting in a total of 18 samples per temperature. These ground samples from the periosteal surface of the bones were then analyzed on a Nicolet 5700 FTIR-ATR at an optical range of 2000cm^{-1} to 400cm^{-1}. Principal Component Analysis (PCA) was performed on all possible absorption peak ratios of the normalized spectra and determined the following ratios to be the most powerful to discriminate between different burn temperatures: CI=(565cm^{-1}+605cm^{-1})/595cm^{-1}, CO/P=1650cm^{-1}/1035cm^{-1}, CO/CO_3=1650cm^{-1}/1415cm^{-1}, CO_3/P=900cm^{-1}/1035cm^{-1}, Phosphate High Temperature (PHT)=625cm^{-1}/610cm^{-1} as well as the Line Width, which is defined as the full width at half the maximum of the phosphate peak at 1035cm^{-1}.

PCA also determined that combinations of different ratios are appropriate for different temperature ranges; low temperature burnings (<400°C) are best identified using C/P, CO/CO_3, CO_3/P, CO/P, and the Line Width, temperatures in the middle ranges (400°C –700°C) by the CI and the Line Width and high temperature ranges (>700°C) by the PHT as well as the C/P ratio.

Subsequently, Linear Discriminant Analysis (LDA) was performed based on the calculated indices of each of the spectra. It was found that the rate of accurate temperature prediction within a range of +/- 50°C was 82% in experimental burnings, which is a significant improvement when compared to the 19% accuracy achieved when solely using the CI, as it has commonly been done in the past. These findings are very promising for the advancement of burned bone analysis.

Burnt Bone, FTIR, Crystallinity Index

H100 Patterns and Timing of Cervical Vertebral Ring Epiphyseal Union in Individuals Aged 10 – 30 Years at Death

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After attending this presentation, attendees will have garnered new information concerning the timing and progress of union of the superior and inferior cervical vertebral centra or "ring" epiphyses as it relates to age at death, and as it varies by sex and population. The goals of this presentation are to: (1) provide an understanding of the progress of union of the superior and inferior epiphyses of the centra of cervical vertebrae; (2) explain the correlation between cervical vertebral ring epiphyseal union and age at death; and, (3) report and discuss findings from tests of sex and population differences in cervical vertebral ring epiphyseal union

This presentation will impact the forensic science community by providing an understanding of the timing and progress of cervical vertebral ring epiphyseal union as it relates to age and may aid in the estimation of skeletal age of unknown individuals, an important aspect of human identification.

For this study, data were collected from a sample of 100 individuals (34 African American females, 19 European American females, 21 African American males, 26 European males), aged 10 to 30 years at the time of death, from the Hamann-Todd Skeletal Collection. Eleven sites

of cervical vertebral ring union were observed: the inferior surface of the second cervical vertebra (C2) and the superior and inferior surfaces of C3 through C7. A modified five-stage scoring method was used: Stage 0, no union; Stage 1, beginning or progressing union; Stage 2, fully fused epiphyses but lacking in remodeling, thus revealing grooves throughout the centrum; Stage 3, full fusion with a groove in some areas and remodeling in other areas; and Stage 4, full fusion with complete remodeling throughout the centrum.

There was a strong correlation between mean values for vertebral ring union in the total sample (r=0.78). Females showed a higher correlation between vertebral ring union and age at death (r=0.83) compared to males (r=0.73). European females showed a slightly higher correlation between vertebral ring union and age at death (r=0.89) than African American females (r=0.83), while African American males showed a considerably higher correlation (r=0.76) compared to European American males (r=0.49). Student's t-test results indicated no statistically significant differences in mean epiphyseal union values between females and males with populations combined, or between African American and European American females; however, the difference between African American and European American males was significant ($p<0.05$).

Raw data observations showed that more cranially oriented epiphyses seemed more advanced in union than caudally oriented epiphyses. A student's t-test yielded a statistically significant difference between the inferior ring epiphysis of C2 and that of C7 ($p<0.01$), with C2 being more advanced than C7; yet, there was no statistically significant difference between C2 inferior and C7 superior. Epiphyses were divided by cervical vertebra type—mean epiphyseal union values for C2-C3 were compared to C4-C5 and C6-C7—and an Analysis of Covariance (ANOVA) test showed no statistically significant difference in the progress of union according to vertebra type. Further, age at death correlated similarly across vertebra types: the correlation between age and C2 – C3 epiphyeal union mean values was r=0.74, for age and C4 – C5 r=0.76, and for age and C6 – C7 r=0.78. Epiphyseal union mean values of the superior centra were compared to the inferior centra for each vertebra; results indicated no statistically significant differences.

Observational analyses of the raw data were as follows: in some females and males, aged 10 – 12 years, union had already begun (Stage 1). Stage 0, no union, was surpassed by 19 years in females and 21 years in males. Stage 1, union in progress, remained evident in some males at 23 years and 19 years in most females, except for one female aged 30 years, who was thought to be an outlier. Stage 2, complete union with grooves, was first evident at 13 – 14 years for females and 15 – 16 years for males. Stage 2 remained evident in males at 25 years and in what was believed to be an outlier female aged 30 years. Stage 3, complete union with both grooves and remodeling, was first seen at 15 – 16 for females and 17 – 18 for males. This stage remained evident in both females and males aged 30 years. Stage 4, complete union with complete remodeling (no grooves), was first seen in both females and males at ages 17 – 18 years.

No individuals in the sample displayed all Stage 4 union. This finding may reflect the difficulty in distinguishing a groove from a scar, a slight color demarcation not eradicated by remodeling. In conclusion, cervical vertebral ring epiphyseal union may serve as a useful guideline for age estimation in unknown skeletons.

Cervical Vertebra, Epiphyseal Union, Age Estimation

H101 Detecting Submerged Remains: Controlled Research Using Side-Scan Sonar to Detect Proxy Cadavers

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After attending this presentation, attendees should understand the application of side-scan sonar to forensic water searches, and specifically how this technology is used to detect submerged human remains. Controlled research will be presented that investigates the variables influencing side-scan sonar searches for submerged bodies.

This presentation will impact the forensic science community by explaining how this technology can be utilized in forensic water searches and the best parameters to employ when searching for a submerged body.

Searching for submerged bodies in water environments can be a challenging task for investigators. While traditional methods, such as cadaver dogs, dive teams, and underwater cameras are common methods for water searches, geophysical instruments and methods are being integrated into search protocols. The main advantages of incorporating geophysical methods for submerged body searches include: a more thorough search, a considerable decrease in search time, and a reduction in the personnel required to perform a detailed search. As a result, numerous law enforcement agencies have now incorporated side-scan sonar as the initial method for their search protocol. When water conditions are appropriate, this technology is used to locate submerged bodies that are usually then retrieved by divers. While side-scan sonar has become a valuable geophysical tool for forensic water searches, controlled research is paramount to determine the best practices for searches in various aquatic environments. Controlled research provides a structured environment in which to investigate variables that influence the effectiveness of the technology and to provide valuable experience for sonar operators. The purpose of this study was to conduct controlled research, using proxy cadavers, in order to evaluate the applicability of side-scan sonar technology and search methods. In addition, the best practices for employing this technology in forensic searches in freshwater ponds and lakes, in a humid, subtropical environment in Central Florida, were developed. Three pig carcasses (*Sus scrofa*), utilized as proxies for human bodies, were staked to the bottom of a large borrow pond, on a flat, sandy bottom. This set of pig carcasses represented medium-sized adult bodies (51 – 54kg). The specimens were monitored for a period of 81 days, which resulted in nearly skeletonized and disarticulated bodies. A dual frequency (900/1800kHz) side-scan sonar unit (deployed from a pontoon boat) was utilized to monitor the pig carcasses, and divers photographed the carcasses during each data collection event when water visibility was appropriate. Transects were conducted with a 20m swath width using both the 900kHz and 1800kHz frequencies. Results show that this technology successfully located medium-sized proxy carcasses on a flat, sandy lake bottom when experienced operators were conducting the search. In the initial stages of decomposition, the carcass (i.e., the acoustic shadow of the carcass) maintained the morphology of a pig that could be easily discerned. However, in later stages, the decomposing carcass was still detected with an accompanying shadow, but did not maintain the morphology of a pig. As decomposition advanced to disarticulation, the carcass progressed to a cluster of distinct features with little to no accompanying shadow. Also, the optimal towfish frequency was 900kHz because this frequency depicted a more discernible and consolidated carcass when compared to the higher resolution of the 1800kHz frequency. Therefore, in the appropriate conditions, side-scan sonar is an effective tool for locating submerged bodies in freshwater lakes and ponds, in a humid, subtropical environment. However, during the later stages of decomposition and disarticulation, it may be more difficult to locate a body since the disarticulated remains will no longer appear as a

clustered group of features and may be devoid of a discernible acoustic shadow.

Submerged Bodies, Geophysical Searches, Side-Scan Sonar

H102 Preliminary Validation of Handheld X-Ray Fluorescence (HHXRF) Spectrometry for Distinguishing Osseous and Dental Tissue From Non-Bone Material of Similar Chemical Composition

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After attending this presentation, attendees will have a better understanding of the benefits of utilizing Handheld X-ray Fluorescence (HHXRF) spectrometry and statistical analysis to distinguish fragmentary osseous and dental material from non-bone material of similar chemical composition.

This presentation will impact the forensic science community by demonstrating that the HHXRF is a valid method for distinguishing between fragmentary osseous or dental material and non-bone material of similar chemical composition in a laboratory setting.

Identifying bone and osseous material that is highly fragmented, burned/charred, and subjected to extensive taphonomic processes can be difficult based on the poor quality of the bone. In most cases, bone and non-bone fragmentary materials are sorted in the field using gross methods; however, in cases involving indeterminate fragments, other methods may be used to sort the material in a laboratory setting. Current laboratory methods include histological analysis and chemical methods determining elemental composition. A recent chemical method proposed by Ubelaker et al. (2002) involves the use of Scanning Electron Microscopy and Energy-Dispersive X-ray Spectroscopy (SEM/EDS).[1] This method provides the Ca/P ratios of analyzed materials, and allows for the formation of a spectral library. More recently, X-ray Fluorescence (XRF) spectrometry, which analyzes the chemical composition of small, fragmentary specimens, and then allows for the specimens to be sorted based on Ca/P ratios, was proposed as a viable method.[2] HHXRF instruments have recently come into use in areas of criminal justice and physical anthropology.

The purpose of this research was to determine the applicability of using an HHXRF for discriminating non-bone material (including material with a similar composition to bone) from osseous and dental material using statistical methods for discrimination purposes.

A total of 28 samples were analyzed, with three spectra taken from different locations on each sample. Samples consisted of human and non-human bones (archaeological, anatomical), non-biological specimens (rock phosphate, rock apatite, synthetic hydroxyapatite, plastic, glass), and other biological specimens (sand dollar, three shell species, two coral species, turkey spurs, bark). A Bruker Elemental S1 Turbo-SDR HHXRF unit was used with upgraded analytical software, S1PXRF (provided by the manufacturer), which allowed for the detection of low-mass elements. The HHXRF was mounted on a vertical stand for stationary analysis and samples were placed directly onto the examination window for analysis using a 15 kV/Filter 2. Post-processing of the data involved removing calcium from the spectrum and normalizing the integrated area of the remaining trace elements, Principle Component Analysis (PCA) using MatLab version 2011b by Mathworks, and Linear Discriminant Analysis (LDA) based on principle components representing 95% of the variance in the data using SYStat version 13 by Cranes Software International.

Initial analyses indicated that cleaning may be required when soil staining is involved, as a number of dental specimens were discriminated from non-bone samples only after minimal processing with a Dremel tool. Results of the LDA showed 97% average discrimination between bone and non-bone samples (with 2% of bone samples misclassifying as non-bone and 5% of non-bone misclassifying as bone). The samples that misclassified were rock apatite, synthetic hydroxyapatite, and an alligator rib bone. The misclassification was a result of only one out of the three collected spectra per sample, as the other two spectra were correctly classified for these specimens. Statistical methods are being examined to remove possible outliers in the data.

The combination of an HHXRF with statistical analysis shows promise for discriminating fragmentary osseous and dental tissue from other types of material, due to the analysis of all detected elements after removal of calcium from the spectrum and normalization of the integrated area of the remaining trace elements. Further research will explore the application of this method by expanding the sample size and determining which location on the bone offers the most accurate discrimination, as well as the applicability of this method to the field in the form of the establishment of a bone library on the HHXRF.

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Forensic Science, X-Ray Fluorescence, Elemental Analysis

H103 Evaluation of the Oettlé and Steyn Sternal Rib End Aging Method on an American Sample

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After attending this presentation, attendees will have an understanding of the overall accuracy of the Oettlé and Steyn sternal rib end aging technique on an American sample, as well as possible ancestral differences for the method.

This presentation will impact the forensic science community by evaluating if the Oettlé and Steyn technique is applicable to an American Black sample.[1]

Sternal rib end aging was originally developed by İşcan, Loth, and Wright.[2,3] Although their method was developed specifically on White individuals, it was later tested on American Blacks (İşcan *et al.*), in which differences in morphological age-related changes between Blacks and Whites were found, with a general tendency to overage older Black individuals.[4] Additional key features associated with Black individuals were also identified, such as a difference in the scalloping pattern, an earlier development of bony projections, and overall good bone quality, even through old age.

Due to these known ancestral differences, Oettlé and Steyn tested and applied the İşcan *et al*. methods on a South African Black sample.[1-3] Low accuracy rates of the method and a tendency for delayed maturation of South African Blacks were found; therefore, the method was revised to reflect the population. Similar morphological differences observed in the İşcan *et al*. study, as well as plaque-like deposits that formed on the interior pit, were also seen in the South African Black sample.[4]

Ancestral and population-based differences have previously required the use of separate standards in other aging techniques, such as with cranial suture closure; therefore, other aging methods also need to be examined for these possible variations. Due to the presence of known ancestral differences in the aging process of the sternal rib end, the purpose of this research was to determine the accuracy of the Oettlé and Steyn sternal rib end aging method on an American sample, consisting of both White and Black individuals.[1]

In order to evaluate this method, data was collected from the Hamann-Todd collection. The sample consisted of 333 individuals and included male and female right fourth sternal rib ends from both White

and Black individuals, ranging in age from 14 to 80 years. The method was tested on all individuals using both photographs and phase descriptions from the original article.

Accuracy was evaluated through the use of cross tabulation tables, with 34% of the sample correctly assigned to the actual age phase. Additionally, 79% were correctly assigned within one phase, and 94% were within two phases. Spearman's coefficient of rank correlation was also used to examine the relationship between the estimated and actual ages, as well as any differences in accuracy based on ancestry or sex. The overall correlation coefficient for the sample was 0.690, with the highest coefficient of 0.714 for Black males.

Results indicate the Oettlé and Steyn method performs somewhat poorly overall on an American sample, although it is slightly more accurate for American Blacks compared to Whites.[1] Even though accuracy rates were relatively low, results do reflect the need for ancestral and population-based standards. Further research is needed to determine if this method is more accurate than that of other sternal rib end aging techniques, and additional data will be collected from the WM Bass collection to include a more modern sample, as well as to test for possible secular differences.

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Age Estimation, Sternal Rib Ends, Ancestry

H104 3D Methodology Used for Exclusionary Identification at Crime Scenes—Who Was and Who Wasn't Here?

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After attending this presentation, attendees will learn about a morphometric technique for collecting a simple series of intra-hand measurements from photographic evidence and for analyzing these measurements in three dimensions for the purpose of establishing exclusionary identification.

This presentation will impact the forensic science community by showing results from a study to determine inter-observer error for this morphometric technique, expand the scope to include population variability, and examine the comparison of mono- and dizygotic twins to the robustness of this exclusionary technique.

The results of the study presented represent a step toward peer review for the described morphometric technique to broaden its utility in court. This technique was developed for a case in which the defendant claimed his older brother, a convicted child abuser, was the perpetrator of rapes against their 2-year-old niece. The rapes were recorded in a series of photographs that the Federal Bureau of Investigation determined to have been taken on two separate dates and in a videotape that occurred on the same date as one of the sets of photos; there was no DNA or dermatoglyphic evidence collected in this case. The prosecutor sought to add to the list of charges against the defendant by identifying whose hands were depicted in the photos, which for the most part showed an adult's fingertips and the victim's anatomy. The judge allowed the analysis to continue and ruled that the results were scientific in nature; however, he eventually did not admit the results into evidence at trial because the technique had not been peer-reviewed, which could have been a basis for appeal by the defendant.

When photographic imagery is introduced into evidence, one of the difficulties encountered can be the identification of subjects present or absent at the crime scene, absent the gold standard of results from DNA. The measurements of intra-hand landmarks described in this presentation are valuable in that they: (1) are conserved against weight gain and weight loss; (2) become fixed at maturity; and, (3) are easy to take. Such information is currently gathered in hand scanners and analyzed in two dimensions to establish identification for access to secure facilities. These landmarks represent conserved proportional relationships in adults; at maturity (~ 18 years), the growth of the digits, palm width, and other key aspects are established for life. For example, there are now several hundred articles addressing one feature used in our analysis: the relationship of 2nd to 4th digit length, a marker of intrauterine testosterone. Preliminary results from the above-referenced court case suggest that a 3D analysis enables unique identification of individuals. How unique? This presentation seeks to establish that.

The described procedure involves taking standard measurements of the hands in the photographic evidence, scaling them, and then statistically comparing them. This shape analysis relies on proportional relationships which also overcomes problems of scale and parallax. The scaled images are measured and then a Generalized Linear Model (Principal Component Analysis (PCA)) is used. When data are sufficient, the analysis allows differentiation of persons of interest based on the pattern unique to the individuals. In the proprietary report compiled for the prosecution in the child abuse case described, this pattern does not approximate the certainty level commonly derived from identifications derived from DNA or dermatoglyphs; however, the results showed the individual least likely present at the scene was the brother whom the defendant accused ($p<.031$). It is suggested that application of this technique will have broad-reaching implications in both the conviction of the guilty and exculpatory evidence for those not involved.

3D Morphometrics, Exclusionary, Identification

H105 Developing Frameworks for Regional Forensic Taphonomy Research and Practice: A Multi-Regional Symposium

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After attending this presentation, attendees will appreciate the importance of understanding regional taphonomic variation for forensic anthropology research and practice.

This presentation will impact the forensic science community by stimulating research and collaboration to establish, utilize, and refine methods and guidelines for interpreting regional taphonomy patterns, particularly decomposition.

From both a theoretical and practical point of view, forensic anthropologists need to improve their knowledge of regional and microenvironmental taphonomic variation, particularly related to the estimation of postmortem intervals. During the past 25 years, there has been an increase in attention to forensic taphonomy, but the focus has been largely on taphonomic "universals." Because there is a lack of adequate local datasets, researchers in taphonomy have tended to look where the light was best, and borrow guidelines developed in other regions.

With the expansion of forensic anthropology programs, and the proliferation of associated "body farms," there are now opportunities to rectify this research gap. But it should be done systematically and in a coordinated manner; this symposium is organized as a first step in that direction.

Attention needs to be focused on a conformation of methods and nomenclature to describe variation along a set of common taphonomic

parameters, so that a more complete, multivariate taphonomic data field, based on ecological variables, can be stitched together. This includes the following examples: common ways to express access to heat, such as Accumulated Degree Days (ADD); humidity, such as Accumulated relative Humidity Days (AHD); and decomposition progress, such as Total Body Score (TBS). It also includes standardizing methods for scene investigation and data collection, particularly: (1) logging heat at the scene; (2) calibration with weather station data; (3) collecting data about solar access (e.g., percent of tree canopy and evergreen versus deciduous vegetation shading the body); (4) noting scavenger access (e.g., identifying the local scavenger guild and signatures of their presence); (5) describing seasonal and sub-seasonal patterns; (6) noting variation in the timing of metamorphosis of local sarcosaprophagous insects in terrestrial cases and in the biology of amphipods in marine cases; and, (7) noting variation in local plant distribution and biology.

In the following symposium, Nawrocki and Latham offer a re-framing of the problem of interpreting decomposition within ecozones, rather than "regions" *per se*, differentiating between "core" processes that are more predictable and "peripheral" processes that are more stochastic and disruptive of core process predictability. Simmons and Moffat review non-human animal experiments done in the United Kingdom (U.K.) over five years to validate the relationship between TBS and ADD across variation in climatic conditions and observers. Carter et al. present an effort to validate a universal formula for estimating postmortem interval across regional variation. Dirkmaat and Cabo emphasize the criticality of taphonomy as an integrating principle within forensic anthropology. Milligan et al. provide an overview of issues and patterns related to northern California; Galloway to the California mid-coast; Connor and France to the Colorado Plateau; Bytheway to an arid Texas environment; Dabbs and Martin to southern Illinois; Woods and Pokines to terrestrial and coastal Massachusetts; Anderson and Bell to western Canadian coastal waters; and Sorg and Wren to northern New England.

The papers in this symposium are offered as still-isolated examples of work being done in the U.S., Canada, and the U.K. Work needs to be done via publications and professional conferences, and funded research, in order to cultivate this growing edge of the field. It is interdisciplinary and will require cooperation among disciplines and regions. A proposal is made in this symposium for creating a Regional Forensic Taphonomy Network (regionaltaphonomy.net) as a framework for taphonomy research.

The symposium represents early steps rather than conclusions. There are programs that are not represented, but which are hereby invited to participate in the Network as it progresses forward.

Taphonomy, Regional Variation, Decomposition

H106 Modeling Core and Peripheral Processes in Human Decomposition: A Conceptual Framework

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After attending this presentation, attendees will understand the difference between: (1) systemic "core" ecological processes that drive soft tissue decomposition and on which the prediction of the postmortem interval is based; and, (2) more stochastic "peripheral" phenomena that can significantly deflect the modal decomposition process and thus introduce error into the prediction process.

This presentation will impact the forensic science community by encouraging forensic taphonomists to replace their regionally specific decay models with a more systemic perspective that emphasizes the underlying continuity of decay across different ecozones.

The decomposing corpse is a complex mini-ecosystem. Nutrients and raw materials that once sustained the living body become available to a host of micro- and macro-organisms, including bacteria, fungi, molds, and invertebrates. The chemical and biological processes that drive this biomass conversion (autolysis, enzyme activity, bacterial replication, and insect growth) all depend, in part, on temperature. As ambient temperature increases, chemical and biological reactions occur more rapidly. As a result, the corpse releases energy and molecules into the surrounding environment, to be exploited by other organisms. The result of this process is soft tissue decay.

The relationship between temperature and insect growth was well understood by at least the 1940s. Although anthropologists recognized that human decomposition occurs more rapidly in hot environments, historical research focused on defining qualitative "stages" of human decomposition that could be tied only loosely to postmortem intervals. Over a decade ago, the University of Indianapolis began to explore the relationship between accumulated temperature and soft tissue decomposition. In three major studies, accumulated temperature explained 73% to 87% of the observed decomposition.[1-3] Time since death fared worse, explaining only 45% to 65% of the observed variation. Despite being drawn from different regions of the U.S., the three samples display remarkably similar decomposition curves. Each curve has a steep initial portion representing wet decay driven by active insect and bacterial breakdown, followed by a plateau where decay slows considerably and the tissues dehydrate. The curves are so alike that the initial equation describing the cross-sectional human sample requires no further modification when subsequent longitudinal pig and human datasets are applied.[1-3] Seasonal variation slightly alters the slopes of these curves and their plateau points, with cool-weather curves being flatter and hot-weather curves being steeper, but seasonality itself does not seem to change the essential form of the curves.

In these studies, accumulated temperature and the dependent biochemical and metabolic processes emerge as the primary determinants of systematic soft tissue decay. When one accounts for temperature, most if not all of the differences observed in the rate of decomposition in different latitudes and regions disappear. It is useful, then, to treat temperature-dependent organismal and biochemical processes as "core" processes that drive decomposition. Because there is a systematic, mathematical relationship between accumulated temperature and decay, methods for predicting the postmortem interval must rely primarily on these core processes. Tables that attempt to relate observed decay status directly to time intervals without incorporating temperature should be abandoned, and studies purported to display regional differences in decay rates should be re-evaluated if they did not explicitly control for differences in temperature.

Clearly, factors other than temperature and invertebrate necrophagy, such as an abundance or absence of water and vertebrate scavenging, can significantly affect decomposition in specific cases. These factors, however, are qualitatively different from core metabolic processes that vary systematically with temperature. It should be difficult, if not impossible, to quantify the effects of these more stochastic variables in most environments, and it is likely that they vary much more randomly across short distances within ecological zones or geographical regions. At best, postmortem interval predictions can be informed by, but not based on, these "peripheral" processes.

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Decomposition, Temperature, Taphonomy

H107 The Taphonomic Revolution: Taphonomy as an Integrating Principle in Forensic Anthropology

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After attending this presentation, attendees will have a better understanding of the significant role forensic taphonomy plays in redefining the field of forensic anthropology as a scientific discipline.

This presentation will impact the forensic science community by outlining the components of forensic taphonomy and how they provide a strong conceptual framework and new lines of research for forensic anthropology.

As originally conceived, and perpetuated for several decades, forensic anthropology was focused almost exclusively on the practical task of victim identification. This perspective provided the driving force behind the development of the field and its professional practice as it is understood today. However, this myopic focus on only the applied nature of the proposed task also imposed severe restrictions on the scope of forensic anthropology and its growth as a scientific discipline. In particular, the paradigm sprung from the classic definition largely failed to produce a cohesive, distinctive conceptual framework capable of supporting strong theoretical foundations or promoting clearly defined lines for basic research.

This presentation discusses how the emergence of forensic taphonomy in the last two decades has come to provide a much-needed theoretical framework, representing not merely a subfield within forensic anthropology, but the conceptual scaffold supporting the discipline and directing the development of the field, especially, the progress of the rapidly evolving subfields of forensic archaeology and skeletal trauma analysis.

The classic laboratory-based, osteological paradigm has focused narrowly on the diagenetic and bone-modification aspects of forensic taphonomy (mostly to assess bone trauma or diagenetic alteration); however, modern forensic applications are increasingly related to other classic taphonomic issues, such as site formation processes or quantitative taphonomy estimates. In turn, these issues serve to outline clear basic research subjects, such as bone transport and transport potentials, bone alteration by a wide variety of agents and natural factors, or anatomical part representation biases.

Moreover, forensic contexts actually offer a unique crow's nest for the observation of early taphonomic processes in real settings, allowing for powerful actualistic studies (this is currently one of the main Achilles' heels of taphonomic research at paleontological settings).

It is concluded that forensic anthropology would benefit from a stronger integration of forensic analysis (and not only taphonomic analysis, but also taphonomic theory), both in professional practice and when designing research. A forensic investigation is better understood and more meaningfully implemented, and communicated, when it is viewed as a taphonomic analysis, thus integrating scene and osteological information within a meaningful conceptual and methodological framework. This also has implications for the training of future generations of forensic anthropologists, who will need to be well-versed and experienced in field recovery techniques and paleontological theory.

Forensic Taphonomy, Forensic Archaeology, Forensic Anthropology

H108 Microbial Community Change Associated With Decomposing Corpses

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After attending this presentation, attendees will understand the basics about microbiome research (study of the genes of microbes) and how recent advances in sequence technology have allowed microbial ecologists to characterize the vast, but measurable, microbial diversity present in animal hosts (e.g., humans) and environmental habitats (e.g., soil). Attendees will be presented with results from recent experiments in which microbial community change was assessed in and on corpses and in their associated gravesoil over the time course of decomposition.

This presentation will impact the forensic science community by revealing the potential of microbial succession during the decomposition process to help: (1) determine the time since death; and, (2) locate clandestine graves.

Biotic signatures of corpse decomposition, such as chemicals or the succession of insects, are commonly used to determine the postmortem interval and to detect gravesoil, but no method is successful in every scenario. Therefore, the development of new forensic tools is important. Microbes are ubiquitous in the environment and they play a key role in regulating the speed of decomposition. However, microbial communities are not currently utilized to their full potential as a forensic tool. Testing whether changes in microbial communities are predictable over the timeline of decomposition is crucial for assessing whether microbes can be used as a "clock" to assess time since death.

Powered by advances in culture-independent microbial community analysis methods and sequencing technologies, recent research has revealed that microbial communities are quantifiable and predictable across habitats such as human skin and soil. In the research presented here, a laboratory experiment was conducted to characterize temporal changes in the microbial communities associated with mouse corpses as they decomposed on soil at ambient temperature for more than a month. Samples from the abdominal cavity, skin, and gravesoil were collected at regular intervals from five corpses. Partial 16S and 18S ribosomal RNA genes were sequenced using Illumina HiSeq deep sequencing technology. Computational pipelines were used to characterize the succession of bacterial and eukaryotic communities during the decomposition process.

Microbial community change was significant and fairly consistent across replicates within each sample site (skin, abdominal, or soil) through the decomposition process. For each corpse-associated site, Proteobacteria increased over time, but most notably in the abdominal cavity—a site in which bacteria of the family Enterobacteriaceae dominated at late stages of decomposition. Furthermore, the decrease in abundance of genera such as Bacteroides and Lactobacillus in the abdominal cavity over time lends support to the long-held hypothesis that decomposition in the abdomen shifts from anaerobes to aerobes or facultative anaerobes after corpse rupture occurs. Importantly, the microbial diversity of gravesoil was significantly modified by corpse decomposition. Several genera from the families Enterobacteriaceae and Rhizobiaceae may be important indicators of gravesoil. Eukaryotic sequence data revealed that nematodes of the family Rhabditidae dominate the meiofauna of both the corpse and soil during late stages of decomposition. Nematodes of the family Rhabditidae may also serve as an indicator of gravesoil. Results demonstrate that microbial

communities hold great promise as a forensic tool as there is a predictable succession over time and taxa are detected.

Microbiology, Decomposition, Ribosomal RNA Genes

H109 An Investigation Into the Relationship of Postmortem Interval and Bacterial Metagenomics of Bone

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After attending this presentation, attendees will gain an understanding of the relationship between Postmortem Interval (PMI) and bacterial metagenomic profiles recovered from human skeletal remains.

This presentation will impact the forensic science community by providing baseline data for exploring the relationships among bacteria, cadaver decomposition, and estimating PMI.

Microbially mediated decomposition of a corpse leaves signatures in bone and their deleterious effects on structural integrity of bone have been defined previously.[1] The amount of bone destruction due to microbial activity has been loosely correlated with PMI, which has been observed occurring later than five years after death.[2] Yet bacteria are involved in postmortem processes immediately following death.[3] Thus, the goal of this research is to produce a community survey of bacterial phyla present in bone of varying PMIs.

Previous analyses of microbial concentration in bone from the University of Tennessee Anthropology Research Facility (UTARF) demonstrated an inverse relationship between total bacterial load and PMI.[4] This study used the same skeletal tissue to determine the bacterial composition of those samples. In doing so, the relative contribution of specific bacterial phyla was expected to change with advancing decomposition, as the sampled bodies transitioned from a high-quality to a low-quality resource.

Eleven human ribs sampled from as many actively decomposing corpses were analyzed. The corpses spanned a PMI of 1 – 48 months. Three soil samples with no known history of human decomposition were also analyzed in order to compare bacterial communities between decomposing bone and "normal" soil. Total DNA was extracted and next-generation sequencing was used to amplify and sequence a 200-base-pair product of the universal eubacterial 16S rRNA gene.[5,6] Recovered sequences were trimmed, aligned, and classified using the Ribosomal Database Project (RDP) pipeline and classifier.[7,8] Identified phyla were analyzed using cluster analysis on standardized abundance data.

Sequences from all rib samples provided 124,164 classified sequences. Results indicated consistency in the presence of specific bacterial phyla. The six most abundant phyla across all bone samples accounted for 94.37% of all classified bacterial phyla. The six most abundant phyla were: Protebacteria (64%), Firmicutes (12%), Bacteroidetes (10%), Actinobacteria (7%), Acidobacteria (1%), and Gemmatimonadetes (0.26%). Nearly 6% (5.62%; n=6,976) of the trimmed and aligned sequences remained unclassified at the bacterial phylum level. Chloroflexi, TM7, and Deferribacteres were consolidated into a single group "other" due to low representation (0.008%). Cluster analysis and MDS mapping on bacterial abundance data identified three significantly different clusters ($p<0.05$). One cluster was characterized by samples 1.2, 2, and 12.3 months. The second group consisted of samples with PMI of 7 – 20 months. The third cluster was composed of the non-UTARF soil samples and the 24- and 48-month PMI bone samples.

In all samples, Proteobacteria were the most abundant and were therefore determined to be of little value as a marker for evaluating temporal trends in phylum-level bacterial community structure. The greatest change in relative abundance occurred among the Firmicutes, Bacteroidetes, and Actinobacteria. The early PMI samples were dominated by Firmicutes. Bacteroidetes dominated the second phase of samples from 9 to 20 months, with the exception of one 12-month sample. The 24- and 48- month samples were dominated by Actinobacteria, and the level of Acidobacteria increased between these two samples, which was consistent with the non-UTARF soil samples.

These results provide baseline data for further exploration regarding the relationships among bacteria, cadaver decomposition, and PMI. Within these data, a succession in the relative abundance of three bacterial phyla was observed, demonstrating the potential for using bacterial metagenomics as a method for estimating PMI of decomposed skeletal remains.

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Taphonomy, Postmortem Interval, Bacterial Metagenome

H110 The Consistency of ADD, TBS, and Decomposition Rate: A Validation Study Spanning Five Years

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After attending this presentation, attendees will understand that experimental studies using Total Body Score (TBS) to predict Accumulated Degree Days (ADD) and decomposition rate have produced consistently accurate results over time.

This presentation will impact the forensic science community by presenting a validation of using the TBS methodology to provide an estimate of the Postmortem Interval (PMI). This method will be shown to produce consistent and accurate results over a lengthy period of time, using different carcasses and different observers; this validation study provides confirmation that this methodology produces robust and reliable results.

When Megyesi *et al*. introduced the TBS system as a means of quantifying the decomposition sequence, and derived an equation based on observed TBS to predict ADD, this provided forensic anthropology practitioners with a standardized method to estimate PMI.[1] Further research has since demonstrated the utility of TBS in predicting ADD and producing PMI estimates under a variety of scenarios relating to both intrinsic and extrinsic aspects of decomposition, including: the

size of the body; the condition of the body; the deposition of the body (e.g., surface, water, buried); and what is often loosely termed the "environmental conditions."[2-6]

This study reports on the consistency of results from research performed at the Taphonomic Research in Anthropology: Centre for Experimental Studies (TRACES) facility in the northwest of England from May to August during the years 2007 to 2012. Data collection protocols at TRACES have remained consistent throughout this period of time; visual observation, body scoring, and photography were undertaken every 50 ADD (as recorded by both the on-site weather station and self-contained thermocouple/data loggers with each pig). Approximately 100 domestic pigs (*Sus scrofa*) were used as controls over five years of experiments, under variable climatic conditions, and using different observers. Decomposition rates were not significantly different and graphs of TBS against ADD produced slopes that were nearly identical. Not only TBS, but individual body region scores (e.g., head/neck, trunk, and limbs) were significantly consistent at comparable ADD intervals regardless of observer, body, or year and prevailing climatic conditions.

To cite just one example where decomposition rate was not significantly different ($t=0.50$, $df=372$, $p=0.616$), the scores for the head/neck region in 24 control pigs from 2009 were virtually identical to those of 10 control pigs from a 2011 study at comparable ADD intervals:

TBS (2009) = -0.799 + 0.458 x √ (ADD)

TBS (2011) = -0.759 + 0.452 x √ (ADD)

The results of this study provide strong evidence that using TBS to predict ADD is consistent with regard to observers, time, and both intrinsic and extrinsic factors affecting a body—and, hence, a relevant, reliable, and valid methodology in the estimation of the PMI.

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1. Megyesi MS, Nawrocki SP, Haskell NH. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *J Forensic Sci* 2005;50(3):1-9.
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Validation Study, TBS and ADD, Decomposition Rate

H111 The Effect of Outdoor Microclimate on Time to Skeletonization in Clothed and Unclothed Remains in an Arid Southwest U.S. Environment

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The goal of this presentation is to explore the forensic taphonomic effects of clothing and exposure to sunlight, with previous research indicating that varying amounts of sunlight have caused differential rates of decomposition due to preferences in feeding behavior of insects and desiccation of tissue. Effects of these conditions were compared based on the Accumulated Degree Days (ADD) to produce a skeleton among domestic pig (*Sus scrofa domesticus*) subjects in an arid environment.

This presentation will impact the forensic science community by demonstrating decomposition patterns in an arid environment as well as the effect of variation caused by cover on decomposing remains from amounts of shade or clothing. Additionally, this study examines the method of estimating PMI developed by Megyesi *et al*.[1]

Six subjects were placed at a site in Warner Springs, CA, located approximately 1341.12m above sea level, in June 2009, in pairs of one clothed and one unclothed pig inside wire enclosures in three outdoor microclimates: full shade; partial shade; and sun. Observations of the subjects' environment, decomposition progress, and associated arthropod activity were made regularly for 40 days, at which point changes ceased to occur hourly. Subjects were then observed every third day for 14 days, and then once per week for 28 days. Decomposition events were noted, including observations of carcass skin color, bloat circumference, lividity, skin slippage, odor, kill-wound discharge, fluid discharge, skeletal disarticulation, and tooth loss. Site observations included ambient temperature, relative humidity, soil temperature, and atmospheric conditions (e.g., sky appearance). Weather data from microenvironment sites and from the National Weather Service/National Oceanic and Atmospheric Administration weather station in Anza, CA, (19.29km from the study site), were used to Calculate ADD (CADD). The ADD were also Estimated (EADD) according to the Megyesi *et al*. (2005) regression equation. The CADD and EADD were compared by Student's t-test in order to examine the effectiveness of the Megyesi *et al*. (2005) method of estimating ADD by calculating a Total Body Score (TBS). The time to each decomposition event between subjects in each microclimate site, and between microclimate sites, was compared by using a two-tailed Student's t-test and/or two-factor ANOVA without replication.

Results from this experiment indicate that microclimate had a greater overall effect on decomposition rate than clothing. However, clothing did play an important role in shielding from necrophagous insects while they consumed decomposing remains. Subjects in shade and partial shade locations reached the skeletal stage prior to the subjects located in the sun, with the clothed subject located in full shade reaching skeletonization first. Statistical comparisons indicate that time to decomposition events were more greatly affected by microclimate than clothing condition. Weather data from a weather station located in Anza, CA, which is at a similar elevation to the study site, was found to be statistically similar to the data from the microclimate locations. There was no statistical difference between EADD and CADD. In a comparison of EADD and CADD to decomposition events by Student's t-test for unequal variance, there was no statistical difference. Calculations based on this experiment indicate that the regression equations developed by Megyesi *et al*. (2005) are a good estimate of ADD in a more arid environment than the environment for which they were formulated. The regression equations will require further testing in other seasons and climates.

The results of this experiment demonstrate that advances have been made in quantitatively estimating PMI. However, repeated testing and longitudinal studies must be undertaken to increase accuracy in future investigations. It is advisable that investigators use caution in outright estimating the PMI of remains found in direct sunlight in arid environments because of the stark lack of change that occurs following desiccation.

Reference:

1. Megyesi MS, Nawrocki SP, Haskell NH. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *J Forensic Sci* 2005;50(3):1-9.

Decomposition, Microclimate, Clothing

H112 The Contribution of Nuclear Magnetic Resonance to the Study of Bone

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After attending this presentation, attendees will have a better understanding of the use of Nuclear Magnetic Resonance (NMR) spectroscopy in forensic science contexts and particularly forensic anthropology and archaeology. The application of NMR is original for these purposes and gives new information about bone composition and its changes during postmortem decomposition.

This presentation will impact the forensic science community by introducing a novel methodology to human bone study by the analysis of carbon ^{13}C and hydrogen ^{1}H atoms contained in bone, and by the observation of differential modifications in bone chemical components over time.

NMR, well known in medicine thanks to Magnetic Resonance Imaging (MRI), can also make possible quantitative studies of different materials from atomic or molecular viewpoints. NMR has been widely used for *in vitro* biological tissues analysis and provides access to fats, molecular conformation, membranes lipid metabolism, and metabolomic analysis as a diagnostic technique to assess the severity of coronary heart disease by the analysis of human serum. However, NMR spectroscopy has been weakly used for bone analysis. It can indeed provide quantitative information on bones, such as composition of the mineral or organic part, hydroxyapatite characterization, identification of amino acids in collagen, presence of citrate, presence of lipids, etc. For this technique, only 80 – 150mg of bone are required for non-invasive analysis, and no chemical treatment is required. With all these advantages, NMR is a tool that should be investigated in physical anthropology and forensic sciences.

Experimental optimization is accomplished on a Bruker Avance 500 spectrometer (protons and hydrogen isotopes are detected at 500MHz, whereas ^{13}C, one of the observable carbon isotopes, is detected at 125.75MHz) under Magic Angle Sample Spinning at 10kHz. Cross polarization with protons is performed to enhance ^{13}C detectivity. Different samples were used in order to choose the better technical parameters according to each sample: fresh human bone (n=4); pig bone with a Postmortem Interval (PMI) of one year (n=4); forensic human bone (PMI=15 years; PMI=30 years; and PMI=60 years).

Using the optimized conditions, NMR bone spectra provided comparative information among samples, such as the presence of citrate or lipids in modern bones, the differential preservation of citrate and lipids in dry bone according to PMI, the presence of exogenous calcite in archaeological bones, the degradation of collagen and its amino acids as a function of time since death, etc. Collagen was found in all samples whatever the PMI. Bio-hydroxyapatite, the main component of the mineral fraction of bone, evolved with time since death toward the chemical structure of pure hydroxyapatite. Similarly, the fats and lipids component of bone are rapidly lost. Such information could lead to use NMR as an alternative method for bone aging, or at least to discriminate between archaeological samples and forensic samples.

More generally, the use of NMR as a complementary tool in forensic anthropology and archaeology opens up new prospects for the study of bone composition and its implications.

Forensic Anthropology, NMR Spectroscopy, Time-Since-Death

H113 Slow and Steady Wins the Race: The Rate and Pattern of Soft Tissue Decomposition in Southern Illinois

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After attending this presentation, attendees will gain knowledge of the decomposition rate, pattern, and sequence of surface remains over two years in southern Illinois. Scientists and law enforcement officials involved in human remains investigation will benefit greatly from the data and visuals presented, gaining understanding that the currently published decomposition standards are not directly applicable to the regional environment of southern Illinois.

This presentation will impact the forensic science community by instituting a baseline of information for the decomposition of soft tissue in the southern Illinois region. The findings of studies at the Complex for Forensic Anthropology Research (CFAR) will prove more applicable to forensic cases in climatologically and environmentally similar regions than those from any other comparable facility. This location represents a geographic area that is farther north and has a lower temperature than the locations used to create current methods for estimating Postmortem Interval (PMI). Additionally, the soil is extremely poorly drained and acidic which may have some yet-to-be identified impact on the surface decomposition of research subjects.

Twenty pigs (*Sus scrofa*) are being assessed to establish baseline rates and patterns of decomposition at the CFAR. The research subjects were placed on the surface in both sun and shade areas. Three subjects were covered with 18-gauge wire fencing to prevent scavengers from removing them; two were placed inside a chain-link dog kennel; and the remaining subjects were left unprotected. Research subjects were deposited in all four seasons and have been in place for a minimum of nine months. Several have been exposed for two years. Thermochrons were placed at the CFAR to monitor the exact temperature in the specific microclimate at the site for use of accumulated degree days as the method of quantifying the decomposition rate. Observations of the decomposition stage for each subject were collected daily according to the method set forth by Megyesi *et al*. (2005) using the Total Body Score (TBS) for each subject.[1] Motion-activated cameras were used to record still photographs and video of research subjects. The cameras proved extremely useful in identifying the types and activities of avian and mammalian scavengers in the region.

The recorded data show significant differences in both the rate and pattern of decomposition when the CFAR is compared to other facilities. The CFAR has exhibited a range of scavengers, such as the possum and North American Black vulture, which have fed on the subjects throughout the research period. Furthermore, the CFAR has exhibited a slower progression in time between decomposition stages. Compared to previously published standards from east Tennessee, the Sonora desert, and Texas, decomposition in southern Illinois progresses from the “fresh” to “early” decomposition stage at approximately the same time, but then slows drastically. The research subjects at the CFAR stay in the “early” decomposition phase up to 33 days longer (days used to compare to results published before the use of ADD became standard) than in other climates. Additionally, the length of time spent in the “advanced” decomposition stage is longer than previously reported from other regions of the United States. Currently, the fastest time to skeletonization at the CFAR is approximately 20 days. Much of this variation likely results from the lower temperatures observed in southern Illinois compared to those locations from which previous reports have been published. Further research is necessary and ongoing.

Reference:

1. Megyesi MS, Nawrocki SP, Haskell NH. Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *J Forensic Sci* 2005;50(3):1-9.

Taphonomy, Decomposition, Forensic Anthropology

H114 California Central Coastal Morphology, Microenvironments, and Human Decomposition

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The goal of this presentation is to assess the applicability of available temperature data to microenvironment and human decomposition rates.

This presentation will impact the forensic science community by providing guidance as to the methods to interpolate temperature data and uses case studies to show variation in microenvironments.

Accumulated degree days, and consequently decomposition, vary widely within seemingly homogenous environments as seen in case studies and associated temperature data.

The coast of California encapsulates a series of microenvironments in which the average temperatures vary considerably throughout the day and by season. Along the Central Coast, approximately from San Francisco in the north to Santa Barbara in the south, the Santa Cruz and Santa Lucia Mountain Ranges form a coastal barrier with only intermittent gaps. These ranges also block much of the action of the cooling sea breezes, confining much of their action to the narrow bands along the coasts.

In addition to the complication of the mountain barrier, summer months are accompanied by dense coastal fogs which keep the average daily temperatures lower. These thick marine layers form in the late afternoon, remain inland throughout the night, and often only burn off in the late morning of the next day. This fog is a vital ingredient for the thick redwood forests that characterize the coastal regions, providing shade in much of the area.

Inland, including in the valleys between the ridgelines of the mountains, temperatures rise dramatically during the summer, but are also subject to lower temperatures and heavy frosts in the winter months. The summer fogs penetrate less deeply and the redwoods are gradually replaced by larger numbers of scrub oaks. Seasonal variation in average daily temperatures is more extreme in these areas than along the coast.

Analysis of human decomposition in this area has depended primarily on extrapolating from studies elsewhere, the experience of the anthropologists, and on some retrospective studies. The latter have largely been confined to the marine environments. Suggested postmortem intervals are often long, reflecting the lack of comparative information. While additional experimental studies may be helpful in providing guidance, the variation in microenvironments presents an extremely difficult set of parameters.

Using case studies of remains found within various microenvironments in coastal California, the accumulated degree days are shown, along with the total body score, to highlight the importance of localized data on decomposition. Proximity to the coast, position with regard to mountain ridges, local vegetation, and season all present complications. This variation holds even when the interference of local scavengers is excluded. Animals known to consume human remains in these areas include coyotes, raccoon, opossum, wild boar, and rodents.

To utilize accumulated degree days and total body score, relevant data from the recovery area is needed. Weather station data, while distributed throughout California, is rarely adequate for the types of forensic reconstructions needed for presentation in court. Interpolation from known sources can be accomplished and it provides one line of evidence to assist the anthropologist. Recording data from the recovery site in order to compare to the known historical data from a weather collection site is also possible, but here seasonal variation requires long-term comparisons.

Taphonomy, Degree Days, Decomposition

H115 Regional Factors in Central Texas Affecting Postmortem Decomposition in Human Remains

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After attending this presentation, attendees will understand how regional ecological factors of central Texas, including climate and local scavenger populations, affect human decomposition stages and rates and complicate estimates of time-since-death.

This presentation will impact the forensic science community by demonstrating the regional variation in decomposition processes and rates in central Texas, the need for collaborative, multidisciplinary research between regions, and the need for regional models for estimating time-since-death.

Forensic anthropologists and entomologists are often asked to predict the time-since-death for a decedent in medicolegal death investigations. Over the past decades, researchers have worked to develop stages and rates of decomposition that can be used to estimate the time-since-death. However, the process of decomposition is highly dependent on microenvironmental and regional-ecological conditions, making it difficult to apply time-since-death estimations across regions.

Located in the Hill Country of central Texas, the Forensic Anthropology Research Facility (FARF) at Texas State University serves as a natural laboratory for examining decomposition in an ecological zone that is transitional between eastern and western climates. The mean annual temperature is 20.3°C, and the mean annual rainfall is 83.1cm. However, central Texas is known for extreme droughts, extraordinary rainfall events, intense sunlight, and strong winds.

A longitudinal study of human decomposition (N=68 as of July 2012) has been in process at the FARF since 2008. Human bodies donated to the Forensic Anthropology Center at Texas State are placed at the FARF on the ground surface in a supine position, usually under a wire cage that prevents scavenging by medium and large mammals and birds. In many ways, bodies placed at the FARF undergo similar decomposition processes that have been recorded in other geographical regions, but there are several ecological factors unique to central Texas that greatly alter the decomposition rate.

Mummification of soft tissue is one factor commonly observed in central Texas that greatly affects the rate of decomposition. Preliminary results of the study indicate that desiccation of the body by the sun, wind, and soil reduces or prevents putrefaction by microorganisms, which leads to natural mummification. Rapid mummification can reduce the duration of other decomposition stages, such as bloat, and increase the time required for skeletonization. By preventing additional visible changes to the remains, mummification can make estimating the time-since-death difficult. At the FARF, skeletonization can take as little as two months to more than two years depending on the degree of mummification that occurs. Accumulated degree days so far for mummification range from 241 to 1,698.

Scavenging by vultures and other animals is another factor that can greatly affect decomposition rates in central Texas, thus complicating the estimate of time-since-death for forensic scientists working in the region. While vultures are found throughout the United States, the greater population sizes of these birds, and the reduced tree cover of central Texas, makes them extremely important in determining time-since-death. Observations of uncaged individuals at the FARF indicate that it can take between three days to approximately one month for the vultures to begin scavenging the human remains. However, once

scavenging is initiated, an individual in pre-bloat stage of decomposition can be skeletonized in less than 24 hours.

In addition to climate and scavenging, microbial population densities in central Texas may have an effect on decomposition rates. Postmortem interval studies of microbial populations at the FARF have demonstrated there is likely regional variation in the species, or at least population densities, of microbes present on the body after death due to temperature, humidity, and other environmental factors. Preliminary results show that variation in microbial populations can directly affect insect colonization, thus complicating time-since-death estimations.

Ideally, forensic scientists would like to develop a universal model of human decomposition that can be used to estimate time-since-death. However, regional ecological conditions that affect the rate (and possibly stages) of decomposition appear to make this an unrealistic goal. Until forensic scientists truly understand the rates and stages of decomposition, and how they vary from region to region, it is unlikely that they will develop accurate universal or even regional models for estimating the time-since-death. Answering questions about regional variation in decomposition is going to require collaborative research across regions and disciplines with standardized data and collection protocols.

Taphonomy, Decomposition, Time-Since-Death

H116 Regional Taphonomy and Estimations of the Postmortem Interval From Northern California

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The goal of this presentation is to examine the complexity of estimating the postmortem interval in the context of Northern California's varied environments. After attending this presentation, attendees will gain an understanding of the challenges of assessing regional taphonomic processes and the impact of various environments on the active decomposition of human remains.

The presentation will impact the forensic community by highlighting the role of various factors on the distribution of human remains, the rate of decomposition, and the variation seen both between and within regions. These factors contribute to a complex and varied estimation of time-since-death.

In the context of varied microenvironments, estimations of postmortem intervals must consider factors beyond those often considered in controlled studies of decomposition. This presentation will highlight several of the most prominent factors affecting postmortem interval estimations in Northern California. The factors considered include the following: scavenging; fluvial transport; high altitude environments; and, the influence of vegetation growth.

Northern California is both climatically and geographically diverse. The region encompasses the San Francisco Bay Area; the Sacramento Valley and part of the San Joaquin Valley; various mountain ranges, including the Sierra Nevada and the Cascade ranges; large national forests; and, massive waterways, such as the Sacramento and the Klamath Rivers. The climatic zones include coastal environments, Mediterranean zones with dry, hot summers and mild, wet winters, and mountainous environments with substantial snowfall. The varied environments encountered in Northern California provide a unique range of conditions for the recovery and analysis of human remains.

The California State University, Chico-Human Identification Lab (CSUC-HIL) provides search and recovery and forensic anthropology services to most counties in Northern California. On average, the CSUC-HIL is contacted for 10 – 15 searches annually, ranging from clandestine burials to surface-scatter sites. In the last year, these searches have been conducted along several major rivers in Northern California, at altitudes above 7,000 feet, in national forests, in the agricultural areas of the Sacramento Valley, and in coastal areas. Most often, recovered human remains exhibit various taphonomic changes, including exposure to natural elements and scavenging from both small and large scavengers.

Given the diversity of possible environments, the study of taphonomic changes to human remains encompasses a multi-disciplinary approach. The CSUC-HIL incorporates entomologists, pathologists, archaeologists, botanists, and other forensic scientists in scene assessments during recovery efforts. In particular, many of the searches conducted by the CSUC-HIL are complemented by the assessment of vegetation growth rates for the various flora found in Northern California. The use of a forensic botanist in these contexts greatly enhances the ability to narrow the time frame considered for the deposition of recovered remains, as well as the changes to the site of recovery over time. In addition, the CSUC-HIL recovery team often includes a forensic archaeologist when the context of the search is buried remains. The partnership between physical anthropology and archaeology increases the understanding of soil formation and disruption patterns.

This presentation will discuss current and future directions of research related to taphonomic patterns. The prevailing trend has been a steadily increasing case volume for the CSUC-HIL. In light of an increased presence in the search and recovery of human remains in Northern California, research objectives focus on those areas that most affect regional taphonomic patterns. Specifically, studies are focused on examining similar taphonomic factors across multiple microenvironments. These factors include the patterns of fluvial transport, varied access of scavengers in remote sites with dense vegetation or extreme climatic conditions, and the patterns of distribution related to various scavengers native to the region.

Forensic Anthropology, Taphonomy, Postmortem Interval

H117 A Two-Pronged Model for Regional Taphonomic Research: A Case Example From Mesa County, Colorado

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The goals of this presentation are to: (1) characterize expected and observed taphonomic variation in an arid, high-altitude biome; and, (2) exemplify a taphonomic research model using both experimental and historic research paradigms.

This presentation will impact the forensic science community by providing better appreciation for the high-altitude desert as a unique taphonomic environment.

Experimental taphonomic studies isolate and examine variables responsible for differences in the decomposition process. Taphonomic research may also be historic, using past cases and the known taphonomic influences in those cases to better understand the decomposition process. This study is an example of combining the two paradigms.

This study uses both approaches to assess the variability in taphonomic variation in west-central Colorado, including Mesa County, the site of Colorado Mesa University's Forensic Investigation Research Station. Mesa County is on the Colorado Plateau, on the western slope of the Rocky Mountains, and east of the Great Basin. The county includes the largest city on the Rocky Mountain's western slope, Grand Junction.

The Forensic Investigation Research Station was established in 2012 as a taphonomic facility to examine decomposition in an arid, high-altitude desert. The area is at an altitude of approximately 4,600 feet above sea level and receives an average of about 9in of precipitation per year, with very low humidity. Vegetation is xeric, consisting mainly of scattered sage and rabbit brush.

While the Station is initializing a human donation program, domestic pigs are currently being used for research. For this study, beginning in the fall of 2012, a freshly-killed pig is placed outside in the

Station at the first of each month and left outside for one year. This will continue for one year so that a total of 12 pigs are used. The goal is to create a decomposition baseline for the region. Data collected will include temperature, lumens, humidity, entomological data, and a total body score.

The historic approach consists of a review of Mesa County area forensic cases with an assessment of the taphonomy of the remains in each case. The rate and pattern of decomposition in the cases are compared to the baseline data from the facility. Discrepancies between the two will identify variables affecting decomposition that will be added to the baseline study.

The research at Mesa County, Colorado, adds to the growing taphonomic database with information on the arid, high-altitude desert environment. It also adds to extant examples of combining experimental and historical research paradigms in taphonomic research.

Taphonomy, High Altitude, Desiccation

H118 Comparison of Faunal Colonization: Taphonomic Macro- and Micro-Skeletal Changes in Pig Carcasses Submerged in a Deep Coastal Marine Environment During Spring and Fall

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After attending this presentation, attendees will have a greater understanding of the impacts of marine submergence on animal carcasses used as human proxies and on the tremendous potential offered to forensic science by the use of underwater cabled laboratories.

This presentation will impact the forensic community by: (1) illustrating, through photos, video, and micrographs, the effects of deep coastal marine submergence on pig carcasses; and, (2) showing the taphonomic changes found in two different seasons, spring and fall.

The studies presented here are part of an ongoing marine-based, high-resolution, data-rich study of the effects of submergence on faunal colonization, decomposition, skeletal dispersal, and survival, in a variety of habitats, seasons, and depths in the coastal waters of Western Canada. The studies utilize the Victoria Experimental Network Under the Sea (VENUS) underwater observatory which allows for real-time observation using a number of remotely controlled cameras and sensors.

Methods: Two sets of two fresh pig carcasses (*Sus scrofa domesticus*) were placed on the seabed in 2012; the first set was placed in February and the second in August. In both cases, the carcasses were observed using a remote connection to the camera. The carcasses were placed on a custom-built platform directly beneath a video camera mounted on a tripod. One carcass was caged, while the other was fully exposed. The cage bars were spaced to allow sufficient access to large crabs and fish, but small enough to prevent shark access. The platform supporting both carcasses, and the tripod and camera, were lowered onto the sea bed in the Strait of Georgia at a depth of 300m. The video camera was programmed to turn on, with lights, every 15 min and scan both pigs for a total of three minutes. An array of sensors recorded physical and chemical water conditions every minute. These included an oxygen optode recording dissolved oxygen and temperature, a vector current meter, and a CTD measuring conductivity, density, pressure, salinity, Sigma T, and sound velocity. Recording continued for six months. At the end of this time (six months of submersion), the skeletal remains were recovered in order to investigate any surface and microstructural changes using Environmental Scanning Electron Microscopy (ESEM) and sectioned material using Backscattered Electron (BSE) imaging. Sections from several different skeletal elements were embedded in plastic and polished in transverse section for assessment.

Results: In the spring study, the carcasses immediately attracted sixgill sharks (*Hexanchus griseus Bonneterre*) to the area. The cage bars successfully protected the caged carcass and, although the exposed carcass was frequently attacked during the first 24 hours after placement, only a few pieces of tissue were removed. Small (<1mm) amphipods were attracted to both sets of remains within a few hours, although shark disturbance of the exposed carcass prevented settling for 24 hours. After this time, thick layers of amphipods settled on both carcasses to a depth of 2 – 3cm, as well as spreading onto the sediment and cage bars. By the end of Day four, both carcasses were skeletonized and the amphipods had displaced skeletal elements. Other arthropods, such as the three spot shrimp (*Pandalus platyceros Brandt*), were originally attracted to the remains but were competitively excluded by the amphipods. Once the remains were skeletonized, the shrimp returned, as did a variety of crab species. Despite the early shark damage, skeletonization and colonization were very similar in both carcasses. Faunal attraction was greatly reduced by Day 12. A biofilm began to develop on the skeletal elements from Day 28 and extended over the sediment by Day 36. The skeletal elements appeared to degrade, and only the crania were visible by Day 117. The study was repeated in the late summer/early fall. The histotaphonomy from the two pigs will be presented and cross-referenced to the events seen during the exposure experiment, particularly the presence of the biofilm.

Taphonomy, Marine Submersion, Microstructural Change

H119 Underwater Decomposition: An Examination of Factors Surrounding Freshwater Decomposition in Eastern Massachusetts

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After attending this presentation, attendees will have a better understanding of the factors surrounding freshwater decomposition in eastern Massachusetts during the summer.

This presentation will impact the forensic science community by establishing a base set of data for freshwater decomposition in a deciduous forest of eastern Massachusetts. Data collected included the water temperature, ambient temperature, stages of body decomposition, rate of decomposition, invertebrate activity, and scavenger activity.

This study investigated the decomposition of three porcine (*Sus scrofa*) carcasses in the same body of water, under lentic and lotic conditions, and at variable depths. The study was performed in a temperate, mixed forest at the Boston University Outdoor Research Facility in Holliston, Massachusetts, during June and July. The remains were placed in wire dog kennels and positioned at the waterline so they could float or sink and be protected from large scavengers. Two controls were placed in dog kennels and placed in terrestrial environments near the aquatic sites. Data were collected on the invertebrate activity, scavenger activity, water and ambient temperature, stages of body decomposition, and the rate of decomposition for each set of remains. Accumulated Degree Days (ADD) and Total Body Scores (TBS) were used to determine two equations (differentiated by their microhabitat) for potential use in estimating the Postmortem Submergence Interval (PMSI) in death investigations involving similar conditions.

The three aquatic remains floated at the waterline throughout the project until they reached skeletonization on days 16 to 45. The terrestrial controls, in contrast, took 13 to 14 days to reach skeletonization. This slowed rate of decomposition at two of the three aquatic sites was due to adipocere formation, cooler water temperatures, limited access by terrestrial invertebrate activity, fluctuations in water levels, and limited scavenger activity. The third aquatic site, located in the shallow lentic water, underwent

decomposition faster than the other two aquatic sites because it received direct sunlight and underwent extreme fluctuations in the amount of water present around it. These variations had a significant effect on its rate of decomposition, as ambient temperature around the remains was significantly higher than all of the other sites, and mummification occurred rather than adipocere formation.

The terrestrial and aquatic invertebrate activity was extensive both above and below the waterline with 42 families from 17 orders collected and identified. All three sites had similar terrestrial invertebrates including: water striders, blow flies and their respective maggots, and spiders. Dragonflies were prevalent around the lentic sites and damselflies around the lotic site. Aquatic invertebrate taxa that were present below the waterline at all of the aquatic sites prior to and during the decomposition phase included: predaceous diving beetles, aquatic sowbugs, giant water bugs, midge larvae, scuds, dragonfly and damselfly larvae, snails, and leeches.

Through the use of motion detector cameras, the researcher was able to view the activities performed around the remains by vertebrates including: a blue heron, a coyote, a raccoon, multiple black vultures, multiple turkey vultures, multiple squirrels, and multiple adult American bullfrogs. Snapping turtles were present in the lentic environment, but their activity around the remains was unclear.

The information presented in this presentation will be valuable to researchers studying the taphonomic processes that occur in aquatic environments similar to these in the Northeastern United States. An understanding of the invertebrate and vertebrate activity will assist in identifying the cause of postmortem artifacts. The climate, ADD, and TBS data can be combined with existing data to establish a more accurate method for estimating the PMSI.

Taphonomy, Freshwater Variables, Decomposition

H120 Regional and Micro-Environmental Taphonomic Variation and Decomposition in Northern New England

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After attending this presentation, attendees will better understand the potential impact that regional and site-specific microenvironmental factors, particularly temperature, can have on the timing of postmortem processes, and the estimation of the Postmortem Interval (PMI).

This presentation will impact the forensic science community by demonstrating the importance of addressing regional and microenvironmental variation in outdoor forensic cases that do not involve animal scavenging.

Published models for the estimation of PMI generally present a sequential decomposition process that includes insect involvement and no scavenger modification. These models are all based on regionally-specific datasets, which may not be applicable to other geographic locations, or even to different microenvironments within that region.

Important regional taphonomic factors for Northern New England include a colder, seasonal climate, a densely forested landscape, and a high level of scavenger access. Using an experimental pig cadaver outdoor case series that excludes mammalian and avian scavengers, the authors have found that both regional factors (such as seasonal temperature fluctuation) and microenvironmental site differences (such as the amount of a forest canopy and the level of moisture retention) impact the rate and character of decomposition. This research utilizes decomposition phase, Accumulated Degree Days (ADD), Accumulated Humidity days (AH), Total Body Scores (TBS), and percent decomposition to track postmortem change including contextual information about forest canopy and ground vegetation.[1]

Two pig cadavers were placed in cages measuring 1.8x2.4m, which allowed insect access, but excluded most mammalian or avian scavengers. The pig cadavers were placed on the same day in late fall, on the day of death. Wildlife cameras were set up within the cages to capture photographs of the cadavers in 15 min intervals. A weather station recording hourly temperature and humidity data was also placed next to the cages. The cages were placed approximately 30m apart. Each pig was placed in a unique microenvironment, one in an open, grassy meadow (designated “Field Pig”), and the other under an evergreen canopy with a pine-needle forest floor (designated “Woods Pig”). These are typical sites for forensic cases in northern New England. This paper focuses on the time period from placement on November 3, with a subsequent snowy winter, extending to June 30. Complete skeletonization occurred during this time frame.

Observations of these two microenvironments showed that year-round forest canopy significantly impacted the rate of decomposition. The Woods Pig progressed through stages of decomposition at a faster rate than the Field Pig, particularly bloat and the early decomposition phases. The Woods Pig had a higher TBS at all 100-ADD benchmarks, despite lower temperatures and a slower build-up of ADD in the woods. The Woods Pig reached full skeletonization earlier, on June 24 (TBS 29), at 1,151 ADD, and an AH of 18,919. By this date, the Field Pig was not completely skeletal (ADD 1,365 and AH 18,827). The Field Pig reached full skeletonization (TBS 29) four days later, on June 28, at 1,440 ADD and 19,145 AH.

Analysis of ADD and AH showed that the insulating effect of the evergreen canopy not only slowed the progression of ADD, but allowed for greater retention of moisture at ADD benchmarks and sped decomposition. By comparing the trends of ADD and AH for each pig, we found that the Woods Pig had similar AH on the same dates, but consistently higher AH than the Field Pig at the same ADD. Vass presents a formula for an aboveground, universal estimation of PMI that includes percent humidity.[2] The documented results during this study also suggest that factoring in humidity during the estimation of PMI can be helpful in explaining microenvironmental differences seen within various geographic regions.

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Taphonomy, Decomposition, Regional Variation

H121 On Repeatability in Nature and Human Identification

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The goal of this presentation is to ask the scientific audience to re-evaluate the framework that has traditionally been the basis for human identification studies. A bottom-up rather than a top-down approach to studies involving human variation as it relates to identification is suggested.

This presentation will impact the forensic science community by demonstrating the utility of the human ear in image comparison cases and by offering an alternate conceptual framework for human identification.

The impetus for this presentation is a series of questions that arose during the trial of a 2008 robbery/homicide suspect from Michigan. The criminal act was captured on surveillance video and multiple images of the perpetrator’s left ear were clearly visible. Forensic anthropologists at Michigan State University used a dynamic orientation protocol to

capture comparable images of the suspect, morphological analysis, and superimposition to present compelling evidence that linked the offender's ear with the primary suspect in the case.

A question that is typically raised during cross-examination in such cases is, "Are all ears (fill in the structure) different?" One way to answer that question (the empirical, top-down approach) would be to say that no two ears have ever been shown to be exactly alike, based on the samples that have been analyzed. An expert witness could cite Iannarelli who famously is said to have compared some 10,000 ears and found all to be detectably different.[1-4] Then the question becomes, "Just because there were no two identical ears in a sample of 10,000, how do you know there won't be a pair of identical ears in a sample of 20,000 or 100,000?" It is impossible to answer such questions because that would require direct observation of all human ears.

Rather than the traditional empirical (top-down) approach, the question is posed; "Are all ears different?" is better answered with a bottom-up approach, based on the simple and long-accepted principle that nature never repeats itself. No two oak trees, snowflakes, zebra stripe patterns, fingerprint patterns, sets of teeth, irises, or ears are completely identical. Each organism and each component of an organism is produced by interactions among genetics, embryology, fetal development, and post-natal environmental influences. There is a DNA component (certain characteristics run in families); the embryology of any structure is complicated and related to a host of intrauterine variables including the endocrine environment and maternal nutrition; and then, not unlike shoe and tire prints, the anatomical feature will be altered by its continued exposure to an environment.

Of course, the challenge to the forensic community is to detect the anatomical variation that makes all structures unique and present it in such a way that it can be applied to human identification cases. While one scientist may be able to detect differences in some structure, it will be of no value unless the forensic community (and ultimately a lay audience) accepts the conclusion that the variation is individualizing.

There may be no doubt in anyone's mind that the two sets of ear images in the case presented represent the same individual. But can it be proven empirically? In reality, no. Theoretically? Only if nature repeats itself.

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Human Identification, Image Comparison, Ears

H122 Testing a New Computer-Assisted Forensic Identification Method Developed Through Interdisciplinary Collaboration

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The goal of this presentation is to provide attendees with an example of successful interdisciplinary collaboration to develop a new method of decedent identification for routine use in the medicolegal setting. The attendees will receive a detailed account of method testing and results.

The presentation will impact the forensic science community by describing a practical, time-sensitive identification method for decedents associated with a tentative name on arrival to the medical examiner's/coroner's office. The method is responsive to the National Academy of Sciences. Report recommendations and post-*Daubert* evidence admissibility standards because it is replicable and incorporates a quantified error rate.

Decedent identification obtained by a forensic anthropologist through comparison of postmortem and antemortem radiographs is a relatively routine procedure in the medicolegal setting. The determination of consistency in skeletal features is made by comparison of the radiographs based on the expertise of the anthropologist. Computer-assisted radiograph comparison methods have been explored with some success.[1-3] Computer-assisted methods typically work by first determining a score that quantifies how well the shapes represented in two different radiographs match. There are multiple paradigms for using these raw match scores to determine if a correct match has been found.

A computer-assisted radiograph comparison method has been developed for medicolegal decedent identification through interdisciplinary collaboration between forensic anthropologists, biomedical engineers, and software engineers and designers. The paradigm for this method is calculation of the match score for postmortem and antemortem radiographs of the index individual and also for a postmortem radiograph within an array of similar radiographs from random persons of the same age cohort. Early theoretical efforts, pilot study results, and method development were reported at the 63rd and 64th Annual Scientific Meetings of the American Academy of Forensic Sciences.[4,5]

Preliminary testing of the method produced a 0% error rate for comparison of single lateral cervical vertebrae (5 tests of 55 images=100% correct). The error rate for single lateral lumbar vertebrae was inflated by an index image with substantial out-of-plane artifact (15 tests of 30 images=80% correct). When the out-of-plane image was removed, the error rate fell to 0%. For this study, 36 pairs of anonymized lateral cervical radiographs (n=72 radiographs) were collected from a historical archive. Each pair consisted of radiographs of a specific person taken three years apart. All patients had undergone a cervical fusion procedure between the time-points, but only the vertebrae adjacent to the fusions were used. Match scores were calculated for a cervical vertebra (C3, C4, C5, C6 or C7), using the latest time-point to represent the "postmortem" radiograph. The radiograph taken three years earlier represented the antemortem radiograph. In some cases, match scores were calculated for two vertebrae from one person. Prior to calculating match scores, all images were reoriented and magnification adjusted using the QMA® computer software interface (Medical Metrics, Inc.). Match scores were calculated by QMA® for the correct match and also for matches of the "postmortem" radiograph compared to five radiographs from other persons. Different combinations (100) of Region-Of-Interest (ROI) definition, pre-processing filters, and match score algorithms were tested on this data set, resulting in 54,000 match scores to use in optimizing the algorithms. Data were analyzed using effect sizes and Receiver-Operator-Curve (ROC) analysis.

The study resulted in several key findings. Radiograph pairs could be classified as a correct versus an incorrect match in greater than 95% of matches (5% error rate), using the optimized protocol and algorithms in the larger sample. The fine details of higher resolution radiographs can reduce the percent of correctly matched radiographs since the fine details are harder to reproduce from one time-point to another. Improved results were obtained after reducing the resolution of the radiographs to approximately 0.2mm per pixel. A geometrically simple ROI (rectangular) worked well, thus avoiding the need to define a complex ROI (polygon). The reliability of the match algorithms is reduced if significant osteophytes form between the time-points. Using the maximum match score when more than one vertebra is compared between postmortem and antemortem radiographs improves the reliability of the computer-assisted match, an important finding for future systematic testing of skull and chest films. Refinement of the method is

ongoing with further algorithmic optimization testing and inclusion of real postmortem radiographs that may be of different quality than clinical radiographs.

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Identification, Anthropology, QMA®

H123 The Utility of Postero-Anterior Chest Radiographs and the Cervico-Thoracic Junction for Human Identification

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After attending this presentation, attendees will have an appreciation for the accuracy, sensitivity, specificity, and predictive values that postero-anterior chest radiographs offer for identification using osteology of the cervico-thoracic junction (clavicles and the C3 – T3 vertebrae).

This presentation will impact the forensic science community by providing key validation data for the aforementioned methods in respect to a large radiographic sample (1,178 antemortem chest radiographs) and 10 analysts (five trained and five untrained).

Comparisons of Antemortem (AM) and Postmortem (PM) chest radiographs have long been undertaken for human identification purposes. The number of radiographs used in past validation tests has often been small (n<100) and the opportunities for true positive matches limited (i.e., small samples of known individuals with correctly matching radiographs have been used (n<50)). This also applies to recently proposed and standardized methods that employ the clavicles and the C3 – T3 vertebrae. This study aims to redress this situation with a blind test that employs 589 pairs of postero-anterior AM chest radiographs (299 pairs representing the same individual or correct matches) and ten individuals who served as analysts. Five of the analysts were trained in the chest radiograph comparison methods (Joint POW/MIA Accounting Command Central-Identification Laboratory (JPAC-CIL) competency certified), and five analysts had not successfully completed the training and/or were yet to undertake it (untrained). The pairs of radiographs, which were 1940s photoflurographs of military inductees, were sequentially presented on a computer to each analyst, one at a time, with the question: do these radiographs represent the same or different individuals? None of the 1,178 radiographs represented duplicates; that is, radiographs of the same individual were taken by different radiographers on different days. A mask was applied to each chest radiograph to limit the analyst's view of the radiographs to the clavicles and the C3 – T3 vertebrae. Analysts conducted their examinations of the pairs back-to-back wherever possible and a 10 min break every hour was awarded.

Trained analysts completed the radiograph array in a mean time of 206 min (breaks not included; sd=24 min). Untrained analysts completed the array in a mean time of 256 min (breaks not included; sd=97 min). Untrained analysts, therefore, held the fastest and the slowest times. Trained analysts possessed: a mean efficiency (correct classification rate)=0.89; sensitivity=0.93; specificity=0.85; positive predictive value=0.89; negative predictive value=0.92. The total number of correct and incorrect responses for the trained group differed to chance at statistically significant levels (c^2 1074, df=1, p<0.01). The untrained analysts performed slightly worse, mostly due to a higher rate of false positive calls: mean efficiency=0.85; sensitivity=0.86; specificity=0.83; positive predictive value=0.87; negative predictive value=0.88. This result was also different to chance (c^2 =1074, df=1, p<0.01) and different to the results of the trained analysts (c^2 =24, df=1, p<0.01) at statistically significant levels. Two of the untrained individuals who could spare the time to be trained increased their performances on the test two-weeks later by significantly reducing their false positive rate, to call true negatives: mean efficiency=+0.11; sensitivity=-0.04; specificity=+0.27; positive predictive value=+0.19; negative predictive value=-0.02. This degree of improvement was not observed by the four trained analysts who repeated the trial after a two-week period: mean efficiency=-0.03; sensitivity=+0.03; specificity=-0.10; positive predictive value=-0.06; negative predictive value=+0.02.

These results indicate that clavicles and the C3 – T3 vertebrae, visible on AM postero-anterior chest radiographs, can be used as accurate markers of an individual's identity. Untrained individuals can undertake the methods with a high degree of certainty for correct answers; however, trained analysts perform better with fewer false positive responses overall. With training, untrained analysts are observed to improve in the same direction as trained analysts. These results hold significant ramifications for the identification of unaccounted-for individuals from the Korean War, 72% of whom are represented by chest radiographs, buried as unknowns at the National Memorial Cemetery of the Pacific.

Validation Test, Chest Radiographs, Human Identification

H124 Morphometric Comparison of Clavicle Outlines From 3D Bone Scans and 2D Chest Radiographs Using Elliptic Fourier Analysis: A Short-Listing Tool for the Radiographic Identification of Human Skeletons

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The goal of this presentation is to report the performance of a semi-automated computer capability that enables fast large-scale searches of 2D antemortem chest radiograph libraries for potential matches to 3D bone scans. The radiographs of the short-listed candidates can then be compared against postmortem radiographs of the skeleton to confirm, or deny, their "match" status.

This presentation will impact the forensic science community by delivering a method that enables large databanks of 2D antemortem chest radiographs to be quickly searched in reference to 3D bone scans. This holds vital importance for the identification of individuals represented in large assemblages of antemortem chest radiographs, e.g., c.8,100 unaccounted-for U.S. soldiers from the Korean War who were subject to chest radiography at induction into the military.

The visual comparison of postmortem radiographs to thousands of images/individuals in large antemortem chest radiograph libraries, for identification, is impractical in terms of time and cost. Instead, these circumstances demand a computer-automated short-listing tool,

whereby individuals of high match potential can be quickly and reliably isolated for finer and more time-intensive analysis using manual methods. This presentation describes such a method, which uses quantified clavicle shape as the filtering mechanism. The clavicles are useful in this regard because: they are morphologically variable between individuals; are visible on the chest radiographs; and possess high field-survivability in comparison to other osseous elements of the thorax (such as ribs). This study uses 414 postero-anterior chest radiographs (many of which are 1940s 4"x5" photoflurographs) as its antemortem reference sample (note: the clavicle outlines were manually traced on Wacom touch-screens), and uses clavicles from 17 field-recovered skeletons as its test group.

The first procedure necessary for the above-outlined computer-automated comparison is 3D scanning of the clavicles and generation of a surface mesh of the bones (.stl file). The computer then rotates the 3D model and captures regularly spaced 2D images at different orientations. This serves two purposes. First, precise position of the clavicle on any antemortem radiograph cannot be reliably predicted *a priori* so a range of positions is examined; and second, it produces 2D images of the 3D model comparable to the 2D radiographs. From the array of snapshot images, four that cover common antemortem clavicle positions are selected to provide good coverage (the small number reduces the chance for false positive matching). The medial and lateral ends of the clavicles on these four 2D snapshots are then trimmed off as they cannot often be seen on the antemortem radiographs (e.g., shoulders fall beyond the image receptor of the X-ray machine). Both the 2D tracings of the clavicles on the antemortem radiographs and the trimmed 2D outlines of the scanned osseous elements are then subject to elliptical Fourier analysis using 40 harmonics (=160 Fourier descriptors for each clavicle). The shape distance between each of the trimmed 2D outlines and the tracings of the antemortem radiographs is computed by the sum of the squared differences across the 320 Fourier descriptors (left and right clavicle sides combined). The computer then ranks the individuals represented by the antemortem radiographs in order of their shape distances.

Using 17 skeletons of known identity, and tracings of 414 antemortem radiographs, short-lists of the top 5% of the antemortem sample (20 individuals) to include the correct match to the skeleton 74% of the time were found. Short-lists of 104 individuals (25% of the antemortem sample) included the correct match 91% of the time. It took less than 1.5 seconds for each search against the 414 person database. It is concluded that elliptical Fourier analysis of clavicle outline shape provides fast and efficient short-listing of potentially matching candidates from postero-anterior chest radiograph libraries. These short-lists can then be subject to closer inspection using more detailed visual inspection methods, sparing their blanket and time-intensive application to all individuals in the reference library. In the future, this computer search capability will likely facilitate identification of several hundred unaccounted-for U.S. soldiers from the Korean War buried at the National Memorial Cemetery of the Pacific.

Radiography, Morphometrics, Fourier Analysis

H125 Down to the Wire: Radiographic Positive Identification Using Midline Sternotomy Wires

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The goal of this presentation is to report the findings of research investigating the use of midline medical sternotomy wires as a means for positive radiographic identification of unknown human remains.

This presentation will impact the forensic science community by addressing the feasibility of making a positive identification using medical chest radiographs when sternotomy wires are present.

Forensic anthropologists are regularly called upon to positively identify hitherto unknown individuals using comparative medical radiography. Given the *Daubert* criteria for evidence admissibility, and the need for validated forensic methods, this study was conducted to document the statistical validity of employing sternotomy wires as a method of positive identification. This research follows numerous other radiographic studies which have focused on the hyoid, hand, frontal sinuses, vertebrae, clavicle, maxillary sinuses, ankle, and orthopedic surgical devices to identify human remains. While previous studies have tested the reliability of surgically implanted devices, these findings have focused on the type of device, such as hip implants and plates/screws related to fracture repair, or the manufacturer and lot number and serial number of the device. These devices can be useful for refining potential matches, but they are not unique discriminating elements since many individuals have their hip or knee replaced and fractures surgically repaired.

This study is unique in that it evaluates surgically implanted metal sutures for their distinct and individualizing characteristics. To the knowledge of the authors, this is the first radiographic positive identification study in which sternotomy wires have been analyzed. Additionally, this study has the largest participant sample size (46 professionals and students) of any medical radiography comparison study to date.

Sternotomy wires, or sternal wires, are metal sutures used to secure the sternum after open heart surgery or any other surgical procedure in which the sternum is bisected. Sternotomy wires are typically midline along the sternum and can vary in size, shape, and number since they are hand-tied by surgeons. The foundation of this study was the comparison of antemortem and postmortem radiographs with sternotomy wires. The 46 participants were forensic anthropologists and graduate students trained in this identification technique. Participants were asked to match five antemortem radiographs to 20 postmortem radiographs and complete an anonymous survey detailing their education level, degree held, experience making radiographic comparisons, the number of cases they have completed involving radiographic comparisons, and whether they had ever made an identification using sternotomy wires. Additionally, they were asked which aspect of the wires they found most useful in making a match. The chest radiographs with sternotomy wires were obtained from the Michigan State University Forensic Anthropology Laboratory (MSUFAL), the Michigan State University Anatomy Laboratory, the Office of the Chief Medical Examiner in Genesee County, Michigan, and Sparrow Forensic Pathology Services in Lansing, Michigan.

The anonymous survey responses were used to assess accuracy, sensitivity, and specificity. Collectively, participants were 99.5% accurate in correctly matching the antemortem radiographs to the postmortem radiographs. When separated by highest degree held, those with a PhD were 99.7% accurate, those with a Master's were 99% accurate, and those with a Bachelor's were 99.5% accurate. The sensitivity of the 46 examiners was 98.7% and the specificity was 99.7%, indicating very few false positives and false negatives (only two false positives and three false negatives). The majority of participants (65%) found the shape of the sternotomy wire loops the most useful characteristic in determining a match. This was followed by the shape or location of the sternotomy wire ties (26%).

The results of this study demonstrate that sternotomy wires can be used as a reliable method for making radiographic positive identifications. Regardless of a participant's education level or case experience, the presence of sternotomy wires in antemortem and postmortem radiographs yielded high sensitivity and specificity. As the high accuracy rates indicate, sternotomy wires are unique; therefore, the shape, size, and various characteristics of the wires are individualizing and can be used to confidently make positive identifications. This research has contributed a new, statistically acceptable method of positive identification to the forensic anthropology community, and satisfies the *Daubert* standards.

Sternotomy Wires, Positive Identification, Comparative Radiography

H126 Positive Identification Through Comparison of Lateral Patella Radiographs and 3D Scans: A Validation Study

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The goals of this presentation are to: (1) report the findings of research investigating the use of lateral patella radiographs as a means of positive radiographic identification of unknown human remains; and, (2) report the results of quantitative matching of two-dimensional patellae images using Elliptical Fourier Analysis (EFA).

This presentation will impact the forensic science community by demonstrating the accuracy of positive identification utilizing medical radiographs of the lateral patella, as well as demonstrate the feasibility for quantified methods to match 2D images of bone scans with radiographs. Results from these studies will help bring positive identification using medical lateral patella radiographs into compliance with *Daubert* standards.

Two hypotheses were tested: (1) Experienced forensic anthropologists can accurately match lateral patella radiographs; and, (2) a 3D imaged patellae can be accurately matched with lateral patella radiographs using EFA.

The study sample was provided by the Willed Body Program, Michigan State University Department of Radiology.

Radiographs were taken using a General Electric® Amx2 portable X-ray unit. Radiographs were taken according to the standards for radiographic imaging of the patella from the lateral aspect. The distance between the X-ray source and the film was 40 inches, with an exposure of about 60kVp/5mAs. All specimens were radiographed once, then five were radiographed a second time attempting to match the angle of the first image. The first set of images served as simulated antemortem radiographs while the second set served as postmortem radiographs to enable comparisons.

An antemortem pool (n=20) and the five reset radiographs (n=5) comprised the survey radiographs. Images were blocked out with cardstock, leaving only the patella visible. Practicing forensic anthropologists and graduate students in forensic anthropology were asked to match postmortem to antemortem images, or answer "no match." The survey also collected demographic information to analyze the effect of education and experience on accuracy. At the time of abstract submission, 22 completed surveys demonstrate high rates of accuracy (99.5%), sensitivity (98.2%), and specificity (100%).

A Canon® model EOS-40D camera was used to digitize the radiographs on a copy stand with backlighting. Digital images were cropped and equalized in Adobe® Photoshop® CS5. Each patella was then outlined using the Pen tool. The outline was saved as a bitmap (BMP) file for input into SHAPE v1.3.

A NextEngine® scanner digitized each patella's surface in 3D (n=23). A series of 15 2D images were serially captured from the 3D model for comparison to the 2D radiographs. The degree of rotation spanned -15°/+15° around the antero-posterior axis and -10°/+10° around the supero-inferior axis.

The SHAPE v1.3 suite generated Fourier descriptors for all images using 40 harmonics (i.e., 345 2D images generated from the 3D scans and 22 patella radiographs). The sum of the squared differences between the Fourier descriptors for the 3D model and the radiographs was calculated and individuals ranked according to this number. The top-ranked image was accurately matched to the radiographic outline in question in 72% of cases. In an applied situation, creation of a "short list" might be preferred so an expert can then visually determine a match. In this case, note that in 20 of 22 specimens, a correct match was found within the top five of 345 images (1.4%).

Since this sample is relatively homogeneous (mostly elderly White Americans), positive results should be applicable to a more heterogeneous sample; however, increased formation of bony spurs with age may increase heterogeneity. Further studies using EFA should confirm these findings, perhaps including more young individuals.

This project has demonstrated the uniqueness of the human patella in two ways. The survey results indicate that medical radiographs of the lateral patella are valid for use in positive human identification due to individualizing trabecular patterns, bony spurs, and overall shape. In addition, this study adds to the literature on the value of EFA in positive human identification because the lateral outlines of patellae are quantitatively distinguishable, reinforcing that these structures are unique and valuable in forensic casework.

Fourier Analysis, Bone Scan, Patella

H127 Robber's Personal Identification by Superimposition and Metrical Analysis Between Recorded Images and 3D Photogrammetric Avatar of the Suspect: A Pilot Study

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After attending this presentation, attendees will become familiar with the use of 3D morphometric comparison to seek a robber's personal identification.

This presentation will impact the forensic science community by showing an objective but non-invasive technique for obtaining a robber's personal identification through an analysis of recorded images and 3D photogrammetric avatar superimposition.

In the past few years, the technological advancement in personal identification systems resulted in a large production of scientific studies and an increased production of devices and commercial software. Facial identification through analysis of pictures taken from video surveillance systems still remains a difficult issue from a technical point of view.

Currently, nearly everywhere, there are video systems, webcams, digital cameras, and cell phone cameras that are able to film facial images and easily transmit, share, and store them. As a consequence, a video documentation of a criminal act often exists.

Expert analysis is then necessary to either confirm or exclude a specific individual as the subject depicted in an image. Personal identification, however, may be easy when close-up pictures are available or when the subject presents specific features or defects.

Personal identification based on 3D digital photogrammetry presents a natural evolution of previous research in this field by Parameterized Superimposition (PS). The experience was based on 2D/2D comparison between video frames taken from the surveillance camera system during a robbery and frames of the suspect brought back to the same place the robbery was perpetrated.

In the first phase, recorded images of the robber were studied and improved. Frames with a better view of the robber's face landmarks were chosen. Then, the court took the alleged offender into custody. The suspect was transported to the location of the robbery and placed in the same position as the frames from the video footage. Finally, a quantitative comparison between the image of the robber's face and the suspect's face was carried out. For that to be possible, the crime scene—often a bank or a shop—has to stay closed during the investigation and total cooperation from the suspect is necessary.

The purpose of this study is to test a new technique based on 3D photogrammetric avatar: photogrammetry currently provides the most cost-effective 3D capturing system, being fast, inexpensive, and non-

invasive; the equipment necessary for acquisition is easily transportable and offers high reliability. The technique was demonstrated to be suitable for capturing facial morphology for clinical and anthropological use.

The new technique should overcome the limits of the PS technique, while maintaining the same quality level of objectivity and accuracy. This technique involves four steps:

- **Preparatory Phase**: In which the recorded images of the robber are studied and improved. Frames with a better view of robber's face landmarks are then chosen.
- **3D Acquisition Phase:** During which a 3D photogrammetric avatar of the suspect face is created; this phase only requires four photos which are made simultaneously with a calibrated camera.
- **Superimposition Phase:** Preparatory for the final step, involves a meticulous spatial orientation of the 3D avatar in the same position taken by the offender in the selected frames. A snapshot of the 3D avatar is then taken.
- **Metric Image Analysis:** A quantitative comparison between the image of the robber's face and the snapshot obtained is used. To perform this step, it is necessary to clearly recognize at least five landmarks on the robber's face using suitable software. Landmarks are chosen and allocated on individual images in relation to the spatial position of the faces, depending on the attitude taken by the individual. The same points are marked on the suspect's face obtained at the end of the best superimposition. Repeated landmarking by different observers allows the detection of random errors and controls the quality of the landmarking practice within and between operators, minimizing variability. The absolute and relative distances between the marked points, the perimeters and the areas of the triangles obtained by connecting the points, and the compactness indexes are automatically calculated on both images in the analysis. Two series of five sets of numerical parameters can then be compared.

Promising results of a preliminary study, involving experimental subjects and cross comparisons between them are presented.

Identification, Robbery, 3D

H128 Anthropology and Disease Genetics: A New Avenue of Forensic Identification

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After attending this presentation, attendees will better understand the potential utility of disease-specific genetic databases for forensic identification. Attendees will be introduced to the concept that features not previously considered individuating by forensic anthropologists can in fact become vital parts of the identification process with a little background research and collaboration with scientists from other fields. In addition, attendees will be shown both the methodologies required in associating anthropological and genetic practices in this type of identification, and a pilot example of how this may be employed in a real-life scenario.

This presentation will impact the forensic science community by showing how the need for more avenues of forensic identification to increase the likelihood of positive identification of highly desiccated or skeletonized remains under the purview of forensic anthropologists is important. This presentation will encourage a more productive collaboration between forensic anthropologists and geneticists and allow forensic anthropologists the opportunity to expand their avenues of identification by tapping into, until now, under-utilized resources. The concept of utilizing a specific set of medical databases discussed in this presentation provides a new avenue of research that will expand the tools available to forensic anthropologists and drive new research endeavors into exploring the forensic potential of existing medical databases.

Forensic anthropology, especially osteological analysis, has become vital to medicolegal cases in which remains are highly decomposed or skeletonized. One of the main roles of the anthropologist in such cases is the identification of the deceased through the implementation of identification methodologies and the creation of a biological profile of the remains. Unfortunately, there are still few databases to which researchers can compare their findings to help identify the deceased. Without the ability to compare the biological profiles of unidentified remains to potential matches, it is very difficult to give a name to the unknown remains: however, there are many medical patient databases with extensive areas of information that have yet to be utilized by forensic investigators. Collaboration between genetic and forensic researchers can expand skeletal identification techniques and provide new avenues for individual identification through the use of specific medical databases.

This new technique builds relationships between features of the skeleton and medical diseases through common genetic markers which affect the presentation of both aspects in the individual. The interaction of the genetic elements are analyzed for significance and then tested against a sample population. Given the genetic association between the disease and the bony manifestation, the identification of the feature on a set of remains can allow the forensic anthropologist to direct a DNA study in search of the common marker in the gene of interest. Upon finding the marker, suggesting that the individual does have the disease, the anthropologist can then search the local disease-specific patient databases using the biological profile to advance the identification process by composing a subset of potential matches.

A pilot case study on the application of this technique to the relationship between the eye disease Age-Related Macular Degeneration, which is the leading cause of vision loss in older adults, and the bone disease Ossification of the Posterior Longitudinal Ligament, which is the ossification of a ligament in the spinal column will be presented. It is encouraged that forensic anthropologists, while creating their biological profile, note features that may not be considered individuating and research any connections between these features and diseases.

Identification, Anthropology, Genetics

H129 Dental Ornamentation Among Southwest Hispanic Border Crossers at the Pima County Office of the Medical Examiner

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After attending this presentation, attendees will have a better understanding of the phenomenon of dental ornamentation among some Southwest (SW) Hispanics, and a better ability to predict the geographic or national origin of decedents with varying styles of dental ornamentation.

This presentation will impact the forensic science community by increasing the understanding and identification potential of this rapidly growing demographic of American immigrants. With the recent rise in the number of SW Hispanics living in the United States, forensic anthropologists are increasingly faced with estimating ancestry in a more diverse community of Hispanic individuals. As such, an understanding of dental modification may assist in the process of predicting national, cultural, or socioeconomic affiliations of a particular unidentified decedent.

Over the past ten years, the Pima County Office of the Medical Examiner (PCOME) has examined the remains of over 2,000 individuals either confirmed to be or believed to be undocumented border crossers. A small but consistent portion of those examined have exhibited dental ornamentation to the anterior teeth in the form of metal "windows" (gold or silver lining around the tooth), gold or silver crowns, stars or letters inlayed onto incisors, and thin bars of gold or silver placed mesially between teeth. Because this is likely a cultural phenomenon, the

prevailing hypothesis is that the prevalence and type of such cosmetic dentistry will vary by geographic region of origin.

To address this question, over 1,400 autopsy reports, photographs, and/or dental charts were reviewed from all cases of undocumented migrants examined at the PCOME between 2005 and 2012. Both identified and unidentified individuals were included. For the purposes of this study, "dental ornamentation" refers to individuals who have any gold or silver dental modifications present in the teeth between either the maxillary or mandibular canines. These cases were coded as "present," whereas those without ornamentation were coded as "absent." The individuals who were missing all or part of the dentition postmortem, or when dental ornamentation could not be determined, were coded as "unknown." In addition to the presence or absence of dental adornment, sex and age were noted for all cases, and for identified individuals, the country and state of origin within Mexico, Central America, or South America were collected. For those with dental ornamentation present, further details were recorded regarding the type and location of dental ornamentation. Simple percentages, Chi-Square Tests, and Phi Coefficients were calculated using SPSS 19.

Results demonstrate that out of the 991 SW Hispanic migrants included in this study, a total of 115 (11.6%) individuals had dental ornamentation of some kind. Although a small percentage, the relatively even distribution of those examined between 2005 and 2012 demonstrates that dental ornamentation continues to be a consistent phenomenon among undocumented border crossers. Most importantly for identification purposes, the presence of cosmetic dental restorations is significantly higher among Central Americans (21.1%) when compared to Mexicans (7.8%) (x^2=.000). Additionally, a larger proportion of Central American females had cosmetic restorations (28.5%) when compared to Mexican females (16.7%) (x=.002). Cosmetic dental restorations were also present at a higher rate among Central Americans under the age of 30 years than Mexicans of the same age group (x^2=.027). Most interesting, at least 50% of all individuals with dental ornamentation did not have any associated dental work and the ornamentation was purely cosmetic.

The large percentage of individuals with purely cosmetic dental ornamentation supports the argument that this is a cultural phenomenon, as opposed to being limited to therapeutic intervention. Individuals are making a choice to have a gold or silver adornment on their front teeth. Furthermore, the higher prevalence of dental ornamentation within the Central American sample demonstrates regional variation, which also supports the cultural argument. Although additional research is needed to further clarify regional patterns, these results indicate that dental ornamentation may be used to assist forensic anthropologists in predicting region of origin when comparing unidentified decedents to missing persons, especially when there are a great number of possible matches.

Forensic Anthropology, Southwest Hispanic, Dental Ornamentation

H130 A Comparative Study of Biogeochemical Signatures in Bone From Two Groups: Deceased Undocumented Border Crossers From Mexico and Individuals From the Northeastern United States

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After attending this presentation, attendees will understand the utility of incorporating bone biogeochemical studies into casework involving the analysis of unidentified skeletal remains. This research tests the hypothesis that human bones from geographically distant areas of North America (Mexico and western New York State) will reflect the respective environments of residence. The environmental regions differ based on concentrations of trace elements and lead isotope ratios. Diet differences may be reflected in the contrasting populations, as well as differences in sources of lead exposure and possibly other elements associated with occupation or medical interventions.

This presentation will impact the forensic science community by showing how the methods discussed will assist in the identification of migrant individuals in determination of their foreign origin which will impact the forensic science community.

This analysis provides a picture of the elements and isotopes that offer the most discrimination between disparate geographical populations. If it is possible to narrow a possible area of origin, whether natal or residential, this technique could provide a geographical area of focus for investigators to concentrate their efforts in resolving cold cases and the unidentified. There is a national concerted effort for medical examiners/coroners, police agencies, and families of missing persons to utilize repositories like the National Missing and Unidentified Persons System (NamUs) to associate missing persons and unidentified remains through demographic, personal, medical, and biological information. This effort also encompasses the identification work of deceased migrants along the southern U.S. border. DNA services are offered free of charge to agencies and families with the goal of finding identities for the deceased and unidentified; however, the reality of unidentified remains is that the pool of possibilities is very large and there must be a familiar sample for comparison. Biogeochemical analysis of bone may narrow the target geographic area of the unidentified person, help direct the investigation, and improve the likelihood of making an identification.

This study investigates the elemental and isotopic composition of human bones of 22 deceased undocumented southwestern border crossers with positive DNA identifications (residence of Mexico) and 38 deceased individuals from the northeastern United States (residence of western and central New York State). Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to obtain concentrations of 31 elements and Pb isotope ratios of parietal, femoral, and tibial bone samples from Mexico (parietal N=17, femoral N=14 and tibial N=4; paired samples: parietal/femoral, N=11) and western New York State (parietal bone from autopsy series, N=32; parietal and tibial bone from anatomical gift series, N=6).

A metric Multidimensional Scaling (MDS) analysis was performed for all samples with all elements. This showed separation of the two geographically separated groups (New York vs. Mexico) when all data is considered. The significance of the differences between the groups for each element and Pb isotope ratios was then analyzed. When comparing only parietal samples, there were five elements (Al, Mn, Sn, Zn, Fe) that were significantly different between the New York State samples and the Mexico samples at the 0.01 level and one (Hg) that was significant at the 0.5 level. MDS was then performed on all of the parietal samples and included only those elements that were significantly different between the two groups. This analysis showed even better differentiation of the two groups with the elimination of nonsignificant elements. In addition, the pairing of bone samples from the same individual allowed a comparison of elemental concentration and isotopic ratios between different anatomic bone locations. The New York parietal/tibial pairs showed no significant difference, indicating that either bone would yield similar results. The Mexican parietal/femoral pairs only showed a significant difference in the concentrations of Mn with a p-value of 0.007.

A plot of lead isotopes (208PB/206Pb vs. 207Pb/206Pb) revealed both overlap and differences between the southwest and northeast parietal bones. The NY group clustered around values previously reported for individuals from this region, showing relationship to local soil, inputs from U.S. and Canadian gasoline sources, and local atmospheric ratios. Both groups revealed outliers that may be explained by different sources of environmental and anthropogenic lead.

This research was supported in part by a Lucas Grant from the Forensic Sciences Foundation.

Bone Chemistry, Geographical Pattern, Foreign Migrants

H131 Extending the Biological Profile: Using Stable Isotope Analysis as an Exclusionary Tool in Region-of-Origin Investigations of Unidentified Remains

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After attending this presentation, attendees will understand the application of hydrogen and oxygen stable isotope analysis in forensic anthropology, highlighting how stable isotope analysis can contribute to the biological profile of unidentified remains by tracking the movement of a victim prior to death.

This presentation will impact the forensic science community by demonstrating how an extended biological profile that includes stable isotope data has enormous potential for aiding in the positive identification of unknown individuals.

Although anthropologists have used stable isotope analysis extensively since the 1970s to examine diet, weaning, and migration in prehistory, the technique has had limited application in forensic anthropology, demonstrated by the paucity of stable isotope presentations and publications. Stable isotope analysis offers another tool to the medicolegal community to reconstruct the movement prior to death of unidentified decedents and to exclude possible matches and narrow search areas.

Stable isotope analysis is most beneficial to forensic anthropologists in cases where standard methods of identification are unsuccessful. Although the biological profile typically consists of sex, age, ancestry, stature, and antemortem characteristics, stable isotope analysis of biological tissues can provide additional information regarding migration history, including birthplace, the last decade of life, or the weeks and months before death, depending on the tissue sampled. Previous research has demonstrated the utility of using multiple stable isotopes to identify region of origin and/or dietary patterns in border-crossers' deaths from Mexico, U.S. soldier war-dead from the Vietnam conflict, unidentified individuals from California, and the identification of a U.S. airman's remains recovered in Laos.

The application of stable hydrogen and oxygen isotope analysis of biological tissues to investigate origin is possible because stable isotope ratios of water vary systematically and predictably across landscapes due to environmental factors. These isotope distributions can be visualized using GIS maps highlighting distinct isotopic regions, or isotope landscapes (isoscapes). In turn, the isotopes of biological tissues record the isotopic composition of locally available water. For example, human hair records the isotopic composition of drinking water consumed by an individual throughout the duration of hair growth. Ehleringer et al. demonstrated that predictive models relating human hair stable isotope ratios to drinking water—and thus, geography—achieved an overall 86% success rate between observed and predicted region of origin within the continental U.S.[1]

However, the success of origin predictions based on the analysis of stable isotopes in tissues is dependent upon the quality of water isotope data used in building the foundational predictive isoscapes. Here, the impact of a fine-scale tap water collection scheme within two U.S. states on the performance of a continental U.S. tap water isoscape model originally developed by Bowen et al. was investigated. The collected sample set consisted of 186 tap water samples from several cities throughout California and Oregon. These samples were then used to develop a tap water isoscape specifically for the two coastal states.[2] The new model was then used to predict the isotopic composition of 20 validation samples randomly selected from the dataset that were not used in model generation. The agreement between measured isotope ratios and those predicted using the new isoscape was compared to agreement between measured isotope ratios and those predicted using the previous Bowen *et al.* isoscape in order to judge the accuracy of tap water predictions in the new model generated from samples collected at a finer spatial resolution.[2]

This project highlights the utility of stable isotope analysis in forensic anthropology investigations by demonstrating the application of the technique to tissues that record geographic information and by presenting methods for increasing the reliability of region of origin interpretations based on measured stable isotope data.

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Stable Isotopes, Isoscapes, Biological Profile

I1 Incarcerated Foreign Minors in Italy: How to Treat Them?

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After attending this presentation, attendees will better understand the challenges associated with tending to the needs of foreign minors by staff in Italian prisons. This presentation also looks at various alternatives to detention with the aim of reducing recidivism.

This presentation will impact the forensic science community by presenting the challenges that various juvenile detention centers throughout Italy face when confronting the treatment of foreign juvenile delinquents. In addition, this presentation highlights the importance making the most of limited funding through the formulation of innovative treatment plans aimed at this population.

Foreign minors often have problems integrating into Italian society. The two primary reasons for this are their young age and their different cultural backgrounds. The goal of this study is to provide data regarding the treatment of foreign juveniles in the Italian justice system. It is important to create secondary and tertiary prevention services in order to decrease the likelihood of recidivism, and to promote the minor's integration into the social fabric of their adopted country.

The principal motive for which minors leave their homeland is the search for a new way of life. Persichella wrote of "advance socialization," a term that refers to the expectations of well-being that a foreigner might associate with a particular place. These mental images are often generated by the mass media, as well as by relatives and friends who have already emigrated there. These youngsters are often disappointed upon their arrival in the new country, leaving them in a state of relative deprivation, frustration, and tension, which are significant risk factors for the commission of crimes.

The percentage of foreigners convicted of crimes in Italy has increased over the last decade with respect to the number of convicted Italians. This is true for all crime categories. There are many reasons for this phenomenon. Among them are the conditions in which these immigrants find themselves when they arrive in Italy (e.g., how their previous expectations measure up to the reality of their new existence, how successfully they reintegrate with their families, their ability to enter into the work force, and the influence of both Italian and foreign organized crime groups).

Foreign minors are more frequently placed in jail than their Italian counterparts. This is because it is less likely that they would have access to resources that might allow them to be spared detention (e.g., having a nuclear family, a home, steady employment, etc.). In theory, these foreign juveniles are supposed to have access to detention alternatives, but in reality, they must overcome serious obstacles in order to take advantage of them.

Cultural mediation acts to facilitate relationships between individuals from ethnic minority groups and the associated social services that are in place to serve them. The cultural mediator aids the foreigner in understanding how to behave appropriately, and attempts to sensitize him to the customs and way of life in Italy. The cultural mediator also actively participates at various phases of institutional life, for example, in helping the minor to communicate with juvenile justice officials, and with relatives. In addition, the mediator also aids the family in navigating the Italian legal system.

Some juvenile detention centers (IPMs) report a lack of resources and funding necessary to create appropriate treatment plans. They cite, for example, the under-utilization of cultural mediators who are often marginalized and relegated to the singular role of interpreter. Cultural mediators are typically only available on an hourly basis, and are not considered to be an integral part of the treatment team. Short stays in IPMs are also reported to be problematic: repeated transfers make it impossible for staff to get to know the minor in order to create an individualized education program.

When considering the usefulness of detention alternatives, the study and development of sharable management models that address the real needs of minors in the juvenile justice system are of fundamental importance. Facilitating the social integration process, appointment of a legal guardian when necessary, and providing qualified legal representation are high on the list of priorities. Only through the development of a united partnership can a welcoming network be created: one that is able to pick up on the first signs of trouble that often confront foreign minors.

Foreign Minors, Juvenile Delinquency, Incarceration

I2 Minor Perpetrators of Sexual Crimes: Personality, Coping Style, and Parental Care—Twenty-Three Italian Cases

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After attending this presentation, attendees will more fully comprehend the dynamics regarding sexual violence perpetrated by minors in Italy.

This presentation will impact the forensic science community by presenting some of the challenges that professionals in criminology and legal medicine face when dealing with sexual crimes committed by minors in Italy.

In the field of forensic psychiatry, any sexual behavior that occurs *without consent, without equality, or as a result of coercion* is considered to be abusive.[1] In order for sexual behavior to be considered consensual, certain criteria are necessary: comprehension of the nature of the proposal; an understanding of societal standards regarding sexuality; awareness of the potential consequences and alternatives; the presumption that agreement or disagreement will be respected in the same manner; the decision to engage is a voluntary one; and, that those involved are mentally competent.[2]

Equal relationship refers to situations in which the two people involved possess equal power within the relationship and neither of the two is controlled or coerced by the other. *Coercion* refers to situations where one party abuses authority, offers bribes, makes threats, or uses intimidation tactics in order to win the cooperation or obedience of the other. Sexual conduct during adolescence must not be considered deviant if it involves non-coercive interaction between two peers. It is not always easy to demonstrate coercion, though expert testimony evaluations often focus on this critical aspect. At times, it is also challenging to determine what age-appropriate sexual behavior is, and if the two people involved are, in fact, developmental and/or chronological peers. Studies on the topic hypothesize the inability of the adolescent to recognize the other as different from himself or herself, and the difficulties associated with entering into a sexual relationship with another person where dysfunctional coping strategies are often employed. One pilot study from 2002 revealed that the parents of adolescent sexual offenders most often employ an overprotective-affectionless parenting style.[3] "Affectionless control" parenting style is a risk factor for the development of deviant behavior. Comorbidity rates in

adolescent sexual offenders are high and involve behavioral disorders, personality disorders, and emotional-affective disorders. One recent study revealed that about two-thirds of sexual offenders meet the criteria for personality disorder. Impulsivity is one of the characteristics typical of people who exhibit aberrant and violent behavior.[4] The goal of this study is to evaluate the relationship between personality, parental care, and coping style in adolescent sex offenders.

Methods: Clinical interviews; psychodiagnostic testing (MMPI, Minnesota for Adolescents, A/2; Parental Bonding Instrument (PBI; Coping Inventory for Stressful Situations (CISS; Questionnaire I-R, frustration-aggression by Caprara et al.); and healthcare, psychological, and judicial documentation. The sample studied thus far is comprised of 23 unmarried male adolescents between the ages of 15 and 20 years at the time of the interview. The average age was 17 years ± 1.6 (standard deviation), and almost all subjects were Italian (1 Albanian), and Catholic (1 atheist, and 1 agnostic). All were investigated for perpetrating sexual violence on other minors. The sample was taken from Judicial Juvenile Social Services, which intervenes following the commission of a crime by a minor.

Preliminary results have revealed no particular pathologies on the part of the adolescent sexual offenders who were examined by the authors. The type of parenting style they received appears to have been intrusive-overprotective, which did not allow the adolescent to face typical life challenges, thus impeding his ability to develop coping skills. This type of parenting style includes intrusiveness, enmeshment, encouragement of dependence, and cutting the minor off from the outside world.

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Minors, Sexual Crimes, Personality

I3 Alcohol Use and Juvenile Delinquency in Europe: Results of an International Study

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After attending this presentation, participants will be able to recognize some features of the relationship between alcohol use and delinquency among juveniles, particularly the strength and the characteristics of this association in various cultural contexts and the role of drinking patterns in the etiology of crime.

This presentation will impact the forensic science community by serving as a key aspect for understanding the relationship between alcohol use and juvenile delinquency with the aim of improving preventive interventions.

The existence of a significant correlation between alcohol use and crime has long been acknowledged in the scientific literature. Moreover, alcohol use constitutes a serious public health problem. Several studies have shown that alcohol plays a prominent role in the genesis of deviant behavior, especially among the young.

In order to ascertain the existence of significant relationship among alcohol use and delinquency among young people and to describe the nature and characteristics of such a relationship, a database was set up to record the results of the "International Self-Report Delinquency Study 2." This database was created by selecting a sample of young people (N=57,771) of both sexes, aged between 12 and 16 years, in 25 European countries (Ireland, Sweden, Denmark, Island, Norway, Finland, Austria, Germany, Holland, Switzerland, Belgium, France, Spain, Italy, Cyprus, Portugal, Bosnia-Herzegovina, Poland, Russia, Lithuania, Slovenia, Hungary, Armenia, Czech Republic, and Estonia).

The research focused chiefly on the role of alcohol use and the various modes of alcohol consumption, and sought to analyze the influence exerted by different life situations (age, sex, lifestyle, friendships, and personality traits).

Alcohol consumption proved to be a very widespread phenomenon among young people, particularly among youths who commit crimes and those who are victims of crime. Moreover, it emerged that the abuse of alcohol was the consumption modality most closely associated with both delinquency and victimization. Alcohol use was seen to correlate more closely with the involvement in violent crime than with property offenses. A possible explanation for this could be that, since alcohol exerts a pharmacological effect which simultaneously heightens aggression and blunts certain cognitive capacities, its consumption may play a greater role in the commission of unplanned offences.

The analyses conducted on the relationship between the involvement in alcohol use and various psychosocial factors, such as lifestyle, personality traits (self-control and inclination to violence), and belonging to delinquent youth groups, yielded particularly significant results.

When the results recorded in each country were examined individually, the association between alcohol use and delinquency was confirmed in all geographical settings, despite the considerable social and cultural differences. In particular, it emerged that, in all of the countries considered, alcohol use was more closely associated with involvement in violent crime than with property offenses.

The results yielded by the present study indicate that alcohol use is strongly associated with delinquency among young people in Europe and that so-called "binge drinking" is the consumption modality most closely associated with offending.

In conclusion, alcohol use and delinquency are closely related with one another. The nature and characteristics of these relationships suggest that the associations between alcohol use and delinquency are reciprocal rather than one-directional. Consequently, alcohol use constitutes a risk factor for criminal behavior. Likewise, involvement in delinquency increased the risk of alcohol consumption and, especially, of alcohol abuse.

Alcohol Use, Juvenile Delinquency, Relationship

I4 The Juvenile Psychopath: How Young Can We Diagnose Psychopathy and Is This Even Helpful or Ethical?

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The goal of this presentation is to offer insight into whether the diagnosis of psychopathy for juveniles can be made in light of the developing brain and normal behavior variations in childhood/adolescence. It will then discuss the arguments that favor early identification and treatment as well as those proposing that psychopathy is stable and unamenable to treatment. Finally, this presentation will discuss the ethical issues and implications.

This presentation will impact the forensic science community by fully exploring the identification of psychopathy in children and/or adolescents as it relates to diagnosis, treatment, and ethical considerations.

Psychopathy refers to a clinical construct that includes a behavioral pattern marked with risk-taking, sensation-seeking, lack of remorse for misdeeds, absence of empathy, narcissism, lack of emotionality, problems with impulse control, and involvement of criminal activities.

Looking at this definition, one can see that some of these behaviors are consistent with normal development in children and adolescents. Consequently, it may not be advisable to make a diagnosis of psychopathy for persons other than adults as several of its clinical constructs may be too common among adolescents and reflect a transient developmental state rather than a stable personality trait. However, there have been a few studies suggesting that for adolescents: psychopathy can be measured reliably, the diagnosis is the same as that for adults, and the clinical symptoms are stable across time. This presentation will examine these opposing views to help mental health clinicians decide if psychopathy should be diagnosed in children and adolescents.

Another issue to consider is whether early identification helps to decrease the risk of youth developing into adults involved in the criminal justice system. When looking at children and adolescents, the psychopathy construct may be helpful to differentiate "life-course persistent" from "adolescent limited" antisocial behavior. In theory, the importance of studying these traits will help to identify those persons who are more amenable to intervention and treatment. There have been some studies suggesting that early intervention and treatment is possible for certain components found in the psychopathy construct. Thus, intervention for these at-risk youth may be able to impede the possibility of them becoming further disturbed and engaging in more serious antisocial behaviors. However there is also evidence to show that psychopathy is stable and difficult to modify regardless of the age of the individual.

Finally, the ethical considerations of labeling a child/adolescent as a psychopath will be discussed. Taking into consideration its highly stigmatizing effect, the psychological, social, educational, and legal ramifications should be considered if a child carries this diagnosis.

Psychopath, Diagnosis, Ethics

I5 The Role of Minor Testimony in Child Abuse: Limits of Applicability in Forensic Medicine

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After attending this presentation, attendees will understand the forensic importance of minor testimony in child abuse.

This presentation will impact the forensic science community by discussing the possibility to evaluate capability of children to give testimony using a cognitive interview.

Introduction: Child abuse is an important public health problem for society. For this reason, the present case addresses the topic of sexual abuse against children from the expert's point of view and debates the question of applicability of clinical methodology in the forensic context.

The presentation will discuss the problem of so-called "masked" abuse, where the typical absence of objective physical evidence can make the declaration of the minor the only evidence available to the investigators.

The Case: This case stems from an allegation of abuse that concerned a group of teenagers. The investigated conduct relates to acts that could have been done by an educative figure of reference, who would have involved minors in a game having, among its rules, contact with adult genitalia. In September 2011, the judge (who in Italy is called "*Judge of preliminary investigations*") assigned to the case, appointed a board of advisors including a forensic pathologist, two psychologists, and a child neuropsychiatrist.

Objectives: The experts were asked to assess the testimonial capacity of minors. This operation was carried out through a methodological process oriented to probe the "generic skills" and the "specific skills" of involved subjects *(*Italian Guidelines on the Listening to Child Witnesses).

Methodology:

1. Gathering of evidence in accordance with the rules of the protected hearing of the minor.
2. Analysis of the quality and the accuracy of the statements.
3. Analysis of the basic psychological functions.
4. Analysis of the psychosocial context in which the complaint emerged, with particular reference to:
   - Possible elements of influence among children.
   - Possible elements of influence among adults (parents).

Procedures:

1. Use of the cognitive interview for the gathering of declarative contents.
2. Audio-video recording of interviews carried out within a neutral space (room equipped with one-way mirror).
3. Use of "Reality Monitoring" for the analysis of declarative contents.
4. Preparation of a table to compare the statements of minors.
5. Preparation of a check-list of questions for the parents' interview.

Results and Discussion: Clinical and psychosocial indicators were compatible with both the "generic skills" and the "specific skill" of each examined subject. Moreover it was necessary to partly review predisposed methodology, renouncing the use of the check-list prepared to interview minor's parents. In fact, the lawyer for defense contested the competencies of appointed experts about minor's parents' interview.

This presentation can be useful in considering the paradoxes in the work of the expert, who when called to apply the clinical methodology that makes him competent to carry out an inquiry, may have to readjust the rules of forensic science to the legal setting.

Minor Testimony, Child Abuse, Cognitive Interview

I6 Differential Diagnosis Between Munchausen Syndrome by Proxy and Overanxious Mother: A Case Report

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After attending this presentation, attendees will be able to understand that some particular forms of child abuse such as Munchausen Syndrome by proxy need a careful scientific multidisciplinary approach.

This presentation will impact the forensic science community by demonstrating the importance of a careful approach to diagnosing Munchausen Syndrome by proxy as well as creating an informatics system that allows doctors to check how many times parents bring their babies into an Emergency Department and the medical reason of such admittances.

The term Munchausen Syndrome was commonly used to describe adults who presented themselves with a false illness story. They are affected by psychiatric factitious disorders that some recommend to re-classify as somatoform disorder in the DSM-5.

The definition of Munchausen Syndrome by proxy child abuse is: when an infant or a child is presented to doctors, often repeatedly, with a disability or illness fabricated by an adult, for the benefit of that adult.

In comparison with other forms of child abuse, this is more complex to diagnose because it involves not only a careful pediatric examination of the infant or child, but also a psychological analysis of the parent's behavior. It could be considered "normal" that a mother requires medical assessment and care for a child's presumptive illness; hence, it is never an easy task for the pediatrician to understand that the parent is

fabricating acute signs and symptoms to draw attention to himself or herself, rather than to injure the child.

The difficulty is to assess if clinicians mean that the mother is only a bit anxious about the child's health, or if she has a psychological or psychiatric disease and there is a concrete risk for the child's integrity.

Presented here is a case of a 4-year-old child who during his life was brought to the hospital at least 30 times by his mother with varying complaints. In four years, this baby presumptively manifested: diarrhea, vomiting, nausea, fever, dizziness, vertigo, absence seizures, syncope, abdominal pain, bilateral hand pain, mucus in feces, gastro-esophageal reflux disease, dyspepsia, and pollakiuria. In most of these cases, the symptoms were only reported by the mother who pretended to submit the child to accurate laboratory and instrumental examinations. The physicians did not find any kind of chronic or acute disease in the child.

In the last admittance to the hospital, the mother stated the baby showed minute and diffuse hemorrhages that suddenly appeared in the morning, coupled with bilateral ankle pain. Even in this case, the physicians did not find such signs, but the mother asked them to examine red spots on the skin. For these reason, the baby was submitted to hematological analysis because the mother was totally convinced the baby had leukemia. After four days of hospital observation, the baby was discharged without diagnosing any condition; the physicians recommended psychological consultation for the mother. The mother did not accept this recommendation and showed great anxiety and worry about her child's health. Forensic psychiatric examination was performed and diagnosed the mother's Obsessive–Compulsive Disorder (OCD). These aspects will be discussed in the following presentation, underlining that the diagnosis of Munchausen Syndrome by proxy can be elusive, especially in a young child. Health organization systems need to implement methods for better diagnosing Munchausen Syndrome by proxy.

Munchausen Syndrome, Psychiatric Disease, Forensic Medicine

I7 Forensic Science in the Islamic Legal System

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The goals of this presentation are to: review comparative studies of religious principles and forensic activities; understand Shari'ah, the definitive Islamic law or doctrine, the source of law and moral guidance, the basis for both law and ethics; to learn about the medicolegal death investigation system in the Kingdom of Saudi Arabia (KSA) and its uniqueness, which was exclusively derived from Islamic judiciary based on Shari'ah law; review medical science in the history of the Moslem world, which is important in order to contextualize the applications of current forensic practice; to understand the role of forensic psychiatry in Islamic jurisprudence which can be traced through the Holy Qur'an and Sunnah (the teachings of the Prophet Muhammad); learn about the Islamic legal texts that give a wealth of material on mental illness, or insanity, and its existence in the Islamic literature, including law manuals and precedent/ruling (Fatawa) collections; explore the cultural, religious, and social contexts of practices of discipline and punishment in the Kingdom of Saudi Arabia; understand how disciplinary practices that are considered normative in one culture may seem neglectful or abusive in another; realize the definition of discipline means those practices (verbal, physical, emotional) intend to have children behave in specific, usually culturally normative ways will vary from culture to culture; review recent controversies that range from criminal material support of terrorism cases to civil challenges of policies of motor vehicle departments regarding the wearing of a type of Muslim veil, the hijab, in state identification photographs; and set a precedence among professionals for international understanding and to foster mutual cooperation.

This presentation will impact the forensic science community by ensuring through effective communications of the true nature of the Islamic legal system and the role of forensic science within, a better understanding of a misrepresented group of people, which will foster mutual cooperation among people globally. With this presentation, the basic premises will be presented for the participating audience to gain knowledge of the following: practice of religious principles in daily life at work—not just worship or recreation and family—differs from the secular life style. Yet, people are basically the same in corresponding the need for trust and respect, belonging and proclamation for fairness, setting rules to regulate ethics and morals, and supporting the values of liberty and humanity for everyone. With the many misconceptions of differing cultures and religious practices in people's lifestyles around the world, the audience will hear firsthand forensic science professionals speak on the practical experience in the Islamic legal systems. This session will highlight the Islamic systems in the practice of some medicolegal issues, such as medical ethics, consent, confidentiality, care of patient near the end of life, euthanasia, suicidal matters, and many other interesting aspects under the scope of Islam jurisprudence. Practices of discipline and punishment of children in Saudi Arabia families is highlighted. It is recognized within childhood studies that all societies and cultures bring infants and children into society and culture through a diversity of practices, one set of which is discipline and punishment. This presentation will review the impact of mental illness on patients in the civil and criminal legal domains regarding the application of the field of forensic psychiatry/psychology in the context of Islamic law upon Moslem populations globally. The true understanding of Islam by real people of the faith lends to best practices among experts worldwide. As globalization brings people closer in the workplace, knowing one's colleagues of different nationalities and living principles will enrich the collaboration of efforts in the paramount interest of the consumer world.

With globalization, the world has become home to an increasingly multicultural legal environment in many nations. A progressive legal system in any nation has the capability to understand and resolve the dynamics and the complex legal issues via various forensic sciences.

Cultures vary globally with varying regard for individuality and community distinction. Human beings may seek uniqueness through identity, yet the same people desire a sense of belonging with fairness and equality, regardless of cultural differences.

Different perceptions of values and traditions are based on the lack of knowledge about "The Other." Across the world, people have defined culture-specific traditions and customs, attitudes and values, and ethics and morals. These are based on religious law or civil law, or both combined.

People of westernized and assorted environments tend to have fragmented connections with traditions, religion, and beliefs, and may have a greater acceptance of forensic practices. While Eastern societies tend to have less diverse cultural groups and have more unified traditions, beliefs, and practices surrounding death, for example, they more frequently have religious issues related to various forensic sciences.

Shari'ah is the definitive Islamic law or doctrine, and it is the source of law and moral guidance, the basis for both law and ethics. The medicolegal death investigation system in the KSA is unique in the world. It is exclusively derived from the Islamic judiciary based on Shari'ah law. This is different from other Islamic countries, which have a combination of Islamic and other judiciary systems.

Forensic medicine education in KSA developed in the past few years as a result of the foundation for Saudi specialty certification in forensic medicine. The certificate is a post-graduate qualification equivalent to a doctorate degree in forensic medicine and requires completion of a four-year training program as well as passing annual evaluations and examinations.

This presentation will introduce the role of forensic psychiatry/psychology in Islamic jurisprudence and current forensic practices in the Moslem world. An analysis of the respective histories of national mental health systems, competency assessments and procedures for countries, as well as the larger Moslem world, was conducted.

The impact of mental illness on patients in the civil and criminal legal domains was observed and recommendations were offered regarding the application of the field of forensic psychiatry/psychology in the context of Islamic law upon Moslem populations globally. Additional recommendations were posited for international psychological training and a call for global practice standards.

This presentation will also highlight the practices of discipline and punishment of children in Saudi Arabian families. Discipline means simply those practices (verbal, physical, and emotional) intended to have children behave in specific, usually culturally-normative, ways. It is recognized within childhood studies that all societies and cultures bring infants and children into society and culture through a diversity of practices, one set of which is discipline and punishment.

Most research on discipline and punishment has been done in Europe and North America and has focused on child abuse (violence against the child, which is widely considered to be excessive and damaging) rather than on normative everyday practices, which is the focus of this study.

Modern day people, and some religions, reject the notion that there is an inherent conflict between science and religion. Instead they believe science and religion are two systems of knowledge. Each operating within its own sphere, they are fundamentally in harmony, mutually reinforcing, and both necessary to advance civilization (Baha'i Faith).

Global Understanding, Islamic Jurisprudence, Forensic Sciences

I8 Acculturation and the Use of the Cultural Defense

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After attending this presentation, attendees will be familiar with the literature exploring how levels of acculturation can influence the notion of a "cultural defense." Case examples will be discussed to explore cultural defenses that were used previously in criminal proceedings. The appropriateness of this defense, as well as both the benefits and risks associated with "raising" such a defense, will be explored. Lastly, the presentation will address the role of the mental health professional in cases where culture may factor prominently.

This presentation will impact the forensic science community by showing how the topics covered are important in terms of understanding sentence mitigation in cases where culture is believed to have played a role in criminal offense.

Within the literature, there is much debate regarding the role of acculturation in the implementation of a cultural defense. Acculturation has been a part of the research literature since the 1930s. Over the years, the definition has evolved to describe a fluid-like process in which there is a constant flow of communication between the immigrant and the environment. As this communication transpires, the immigrant is actively internalizing and identifying with symbols of the dominant society. Consequently, the level of the individual's acculturation becomes an integral component in the assessment of a raised "cultural defense." Acculturation may provide a meaningful context from which to explore the behavior of the alleged offender, which may thus impact the severity of his or her criminal culpability.

According to some authors on this topic, a cultural defense should only be allowed for individuals who have yet to assimilate/acculturate to the values and laws of the dominant culture. Past criminal cases such as Kong Pheng Moua, a Hmong man, who abducted and raped a Hmong woman in 1984, under his culture's traditional practice of "marriage by capture," as well as other cases, have shown that culture can be an extremely important variable within the criminal justice system. There are those who question whether the use of a "cultural defense" should be allowed as a viable defense by the defendant. That is, should these individuals who retain their cultural beliefs or aspects of it, and who commit crimes based on those beliefs, be permitted to use their culture as a mitigating variable? On the other hand, there are those who support such considerations in criminal proceedings.

Some mental health professionals have argued that when considering the cultural defense as a mitigating factor, it is important to assess to what extent and degree the defendant's culture played in his or her behavior. If a cultural defense becomes an acceptable defense, what then are the implications for criminal culpability within a multicultural society? Will the nature of the crime be a significant factor? Will the specific type of cultural background of the defendant be a factor?

Most would agree that, while individuals may belong to a group in which there exists certain beliefs or practices, such individuals should nevertheless be held responsible for criminal offenses. However, in light of the undue influence that the person's cultural beliefs may have had, it may well warrant consideration for mitigation in the sentencing phase of trial.

A chief element of the presentation will be to discuss the role of the mental health professional as an expert in these cases, highlighting issues that need to be considered when conducting an evaluation where culture may be of notable importance. When culture is raised as a defense, it is important for the examiner to determine the level of acculturation and the degree to which the individual holds steadfast to the beliefs and cultural practices of his or her group, despite the possibility that the individual's cultural defense claim may be tenuous. Consequently, the complex nature of these cases in which culture may disguise true intent or mental illness, requires the mental health professional to remain sensitive and cognizant of the impact culture has on the cognitive, emotional, and behavioral functionings of the individual.

Cultural Defense, Acculturation, Criminal Culpability

I9 Measuring Preschool Children's Interrogative Suggestibility in Forensic Interviews

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After attending this presentation, attendees will be become familiar with the area of suggestibility in children between three and five years of age. The factors of gender, age, cognitive capacity, and the interval of time between the event and the interview will be discussed.

This presentation will impact the forensic science community by explaining how one of the most important forensic issues during the questioning of a child is the possibility of suggestibility during the interrogation of forensic cases. Ethical professionals must respect and protect childrens' rights (New York Convention 1989; Strasbourg Convention 1996). According to the international literature, childrens' cognitive development is not complete until adolescence.[1-3] Therefore, the problems inherent in children serving as witnesses are self-evident.[4] In preschool-aged children, false memories may be identified because of misinformation and insight bias. Additionally, they are generally susceptible to implanted suggestions.[5,6]

The aim of this study is to verify the possible levels of suggestibility in children between three and five years of age. The variables of gender, age, cognitive capacity, and the interval of time between the event and interview are considered. Ninety-two children were individually examined (44 male, 48 female; M=4.5 years, SD= 9.62).

The most widely used tool for assessing and evaluating levels of interrogative suggestibility are the Gudjonsson Suggestibility Scales (GSS) and the Bonn Test of Statement Suggestibility (BTSS).[7,8] All subjects completed the BTSS (which is indicated for the age range between 4 and 11 years). The duration of this test is about 30 minutes and is structured in four defined phases: presentation of the "toy duck story" with four illustrations, free report of the child, a 15-minute interval for distracting the child, and questioning (31 questions) the child about the story. The BTSS investigates the dimensions: Yield, Shift, Immediate Recall, and Total Suggestibility.[9,10]

The study hypothesizes that the younger children (three years of age) were more susceptible to the suggestibility than the older children (five years of age). Another hypothesis is that a higher score in the

dimension Immediate Recall is positive-correlated with the dimension Total Suggestibility. The last hypothesis is that a high score of Yield is correlated with the younger children and that, in contrast, an elevated score of Shift is associated with older children.

The results of this study of 92 participants concluded: (1) younger children are more susceptible to suggestibility; and, (2) no significant gender difference was observed. The dimension Immediate Recall was negatively correlated with Total Suggestibility (r=-.357).

Social compliance and source monitoring errors can contribute to presenting patterns of suggestibility because older children change their answers more often (Shift).[11,12] Social factors and memory, especially autobiographical memory, can underlie suggestibility. Children will hardly remember their early childhood (4 – 5 years) and it is also known, from numerous studies, that children can lie to avoid punishment or to get a reward.[13-15] In forensic issues, children are normally interviewed several times before a case goes to court. This study shows that repeated questions can transmit the message that they have to change their given answer.

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Eyewitness, Suggestibility, Children

I10 Interrogative Suggestibility in Children: Italian Validation of GSS2 in the Forensic Field

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After attending this presentation, attendees will be able to understand the standardization and validation of the Gudjonsson Suggestibility Scale2 (GSS2) in an Italian research sample. GSS2 is widely used, particularly in preparation of court reports, to derive information on the memory, suggestibility, and confabulation of criminal suspects, victims, and witnesses. The Gudjonsson study was twofold: (1) to present a parallel form of the Gudjonsson Suggestibility Scale (GSS, Form 1); and, (2) to study the test-retest reliability of interrogative suggestibility.

This presentation will impact the forensic science community by introducing a wide sample of subjects compared to the interrogative suggestibility and the study of the correlation among additional variables.

The hypothesis of this paper is that three variables (age, family condition, and type of attachment bond) have a significant association on the degree of suggestibility of children.

The subjects belong to three age groups: six, eight, and ten years of age. The dependent variables are: Immediate Recall; Delayed Recall; Yield 1; Yield 2; Change; and, Total Suggestibility.

Each subject submitted to two psychological tests, GSS2 with Italian translation and Separation Anxiety Test (SAT), to evaluate the way in which the child lives and the living situations (in terms of separation from their parents).

In this study, to investigate correlation measures for the corresponding variables in the two tests, about 100 children were individually examined using 30 variables for each group and all participants completed GSS2 and SAT. The investigation is ongoing. The hypotheses are:

Hypothesis 1 (Age): The higher the age, the higher the scores in Immediate and Delayed recall, and the lower the scores in Total Suggestibility, Yield 1, Yield 2, and Change.

Hypothesis 2 (Children With Divorced Parents): Children with divorced parents have higher scores in Total Suggestibility and Total Confabulation, and score equally in the Immediate and Delayed recall scores and single scores of the Immediate and Delayed recall.

Hypothesis 3 (Different Types of Attachment): Children with Attachment B have a lower Total Suggestibility score and lower total score of confabulation, the same score of Immediate and Delayed recall, which is a very high degree of association; Children with Attachment C have a higher score of confabulation, a higher Suggestibility score, and an equivalent score of Immediate and Delayed recall; Children with Attachment A have a lower score of Immediate and Delayed recall.

The presentation will focus on research related to suggestibility; in particular, time will be given to studies which show how much memory and leading questions may influence the testimony of minors. Finally, the presenters will report on studies which explore the relationship between specific variables and suggestibility interrogative degree and show how memory and testimony may be influenced by different sources of bias which need to be taken into consideration in the forensic field.

In this paper, it is believed that science is now sufficiently evolved so that such interviewing techniques can be applied to interviewing children in child custody evaluations or in cases of alleged sexual abuse.

Test Validation, Impact of Variables, Children Witness

I11 Nonverbal Reaction: Four-Phase Investigation Analysis Protocol

Luca Marzari, BS, Via Tito Livio, 5, Reggio Emilia, 42124, ITALY*

After attending this presentation, attendees will be able to study and identify every possible communicative variable, and verify the coherence of the verbal and nonverbal behavior sequence combinations (COMBO).

This presentation will impact the forensic science community by measuring objective and quantitative parameters during the forensic interview and police interrogation.

The protocol is divided into four different phases and follows rigid rules in the environmental construction of the setting, predisposed with sophisticated instrumentations of audio, video and of biophysiological measurement.

PHASE 1 – This is the phase of harvest dates inherent to the subject (medical anamnesis and structured and semi-structured checklists and includes: social position and personal beliefs, psychological evaluation, self-report list of elements that usually cause anger, disgust, contempt, sadness, surprise, happiness, fear); toxicological analysis and baseline measurement concerning usual nonverbal behaviors modality (use of manipulator's and illustrator's gestures, relax postures, involuntary nervous twitch, etc.) and subject's vocal tone. Video and audio: subject in interaction with an observer.

PHASE 2 – Neurophysiologic baseline measurement, subject verbal stimulation, and recording of potentials correlated with emotional-physiological activations to questions using the 40BSIQ (40 Brain Storm Investigation Questionnaire).

PHASE 3 – Distraction of the subject with intellectual secondary activity.

PHASE 4 – Analysis of the answers and of reactions of the subject submitted to questioning. This phase is the central point of the protocol. The subject is analyzed by four different observers. Everything will be audio- and video-recorded (four cameras, two microphones), and the observers are themselves observed by audio and video.

Nonverbal Analysis, Research Protocol, Observation Setting

I12 Child Pornography: International Cooperation and Legislation in France

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After attending this presentation, attendees will learn about entrapment in France and the different levels of cooperation that permit authorities to catch child pornography suspects worldwide.

This presentation will impact the forensic science community by explaining about the lack of information in this area. This presentation will also demonstrate how getting international cooperation is not a simple endeavor.

Introduction: Child pornography is a form of sexual exploitation of children. The use of telecommunication facilities, particularly the internet, has allowed child pornography to be distributed more widely. The internet is used by pedophiles for four reasons: trafficking child pornography, locating a minor for an assault, initiating communication with a minor, and communicating with other pedophiles. Today, access to pedophilic material no longer requires direct contact between supplier and consumer, but can be obtained anonymously. It is common for pedophiles, and those using child pornography, to be part of virtual groups. They can speak freely about their fantasies and their fears, thus maintaining a good image of themselves while obtaining child pornography material. The existence of these virtual groups has become a major issue, as they are not restricted by territorial boundaries.

Legislation of the Entrapment: Legislation has been passed in a number of countries to tackle the issue. Various police operations have targeted groups of child pornographers. In "Operation Icarus" by Europol in 2011, 269 suspects were identified and 112 arrested in 22 European countries. Since 2007, Article 706-47-3 of the French Penal Procedure Code legislates the actions of "cyber patrol;" however, they are limited in being able to act on this type of evidence. A case illustrates this: the *Cour de Cassation* (Supreme Court) has ruled twice on the facts of downloading child pornography images via an American police website (on February 7, 2007, and June 4, 2008). The *Cour de Cassation* came to explain that, to create a site on the internet, including those created by foreign police, was a violation of the offense. Any evidence gathered through this site is inadmissible, even if there were pre-existing violations. However, the limit also fixed by the *Cour de Cassation* is that if there were pre-existing suspicions, in this case, the challenge would not be cause for cancellation of the procedure, since it would be seen as a provocation to the evidence of the offense (which is accepted), not as a provocation to the offense (which is prohibited).

When states permit it, the police try to use fake sites in order to catch child pornographers. Trawlers and the groomers are among the different profiles of consumers of child pornography. Trawlers are individuals who are looking for sexual material without fixating on pedophilia. Groomers are individuals who seek to attract a minor, using tricks such as falsification of age or adaptation of the speech according to the speaker. The groomers usually have a pretty good understanding of how discussion forums, secure methods of downloads, and exchange of images work. Neither profile is always aware of the illegality. It does not seem difficult to intercept the trawler, who has a low potential to offend, and who does not take special precautions when using the internet. It is more difficult to intercept the groomers, whose computer skills are often high, for which there is a real interest in physical contact, and who would, therefore, have great potential for such offenses.

Conclusion: Most child pornography images are obtained via the internet, which only requires access to a site or to a chat room. Peer-to-peer networks have facilitated the exchange of child pornography, which can explain the development of the phenomenon. The technology gives the offender the opportunity to become a compulsive collector. The internet has encouraged the phenomenon of grooming, which consists of attracting someone and seducing victims. It is important to be able to locate people who use illegal pornography. One way of doing this is to track internet discussions. The principle of loyalty to the evidence can be a source of problems in apprehending child pornographers.

Forensic Psychiatry, Child Pornography, Entrapment

I13 Matricide and Mental Illness: An Italian Case Report

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After attending this presentation, attendees will be given the opportunity to reflect on a case of matricide and the peculiarities of this case presentation.

This presentation will impact the forensic science community by emphasizing a perpetrator's lack of empathy in matricide as a sign of a mental disorder.

Introduction: A case of matricide (which came to attention for an expert opinion as commissioned by the Judge) provided the opportunity investigate this phenomenon and its peculiarities. Matricide, or the murder of one's own mother, has always been considered one of the most abhorrent crimes that can be committed, garnering intense media coverage all over the world.[1] Despite the coverage and furor that cases of matricide generate, the murder of one's own mother is a rare event. In the United States from 1976 to 2005, matricide accounted for less than 2% of all homicides in which the victim-offender relationship is known. This rate is consistent with studies from France, the United Kingdom, and Italy. Selecting only studies on matricide by adult sons, most of the perpetrators were single adult males with an intense relationship with their mother, a lack of interest in other women, a feeling of social inferiority, and an absent or passive father.[2] According to the

literature, the majority of matricidal offenders suffer from severe mental disorders. In particular, matricide seems to be more common among individuals with schizophrenia or other psychoses, to such an extent that matricide was once referred to as "the schizophrenic crime."[3] Among schizophrenic conditions, the paranoid subtype is the most common.[4] Other diagnoses include mood disorders, substance abuse, and personality disorders.[5] Very often, schizophrenic offenders were influenced by psychotic symptoms at the time of the crime. Characteristically, such "psychotic" matricides are committed with excessive force and violence, while the post-offense behavior is non-finalistic and disorganized; concealment of the crime is mostly absent or somewhat mechanical, and the perpetrators usually confess.[6] These offenders often reported feeling that their mothers were either ambivalent toward them or excessively domineering.[7] Matricides are classically committed in the victim's home, usually with a weapon, although asphyxia is also common.

Several schemes have also been proposed to classify the different types of matricidal motives. In his sample, Green reported that the apparent motives were persecutory paranoid (47%), altruistic (24%), or other (29%).[8] In a U.S. study, Hillbrand identified four scenarios: acute psychosis (47%), impulsivity (28%), escape from enmeshment (15%), and alcohol or other substance abuse (24%), with the latter being superimposed upon any of the other three. More recently, Bourget described four leading causes of matricide: mental illness, family abuse by the mother, compassion for the victim, and intoxication.[9,10]

Methods: The body of a 78-year-old woman was found on the floor in her house. Her head had been severely traumatized. Death was due to massive blunt force trauma to the head, possibly using a large hammer that was beside the body at the crime scene. The perpetrator was the victim's 48-year-old son, who lived with his mother and who had suffered from chronic paranoid schizophrenia for more than 15 years. He quickly confessed to the crime, adducing as motive the fact that he was convinced his mother was putting poison in his food, as well as practicing black magic to destroy him. The perpetrator was the third and last child, unemployed, and a bachelor with a very limited social and relational life. The man hit his elderly mother many times with a hammer and then sat in front of her all night waiting for her to die. He later explained that he wanted to be certain she was dead because, after the first blows, he had seen a demon-like vitality in her. He was found not guilty on the grounds of insanity.

Conclusions: In this case, as in others reported in the literature, the perpetrator of the matricide was a schizophrenic with a delusional disorder, and acted extremely violent. The main element of interest is the total lack of empathy with the victim while committing the crime as this is also an indicator of the mental disorder.

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Matricide, Lack of Empathy, Schizophrenia

I14 Emerging Topics in Italian Forensic Psychopathology: Foreign Criminality and Cross-Cultural Assessment

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After attending this presentation, attendees will be able to understand the full importance of developing a model of cross-cultural approaches to the forensic evaluation of mental disease.

This presentation will impact the forensic science community by examining the difficulty recently arising in Italian forensic psychopathology regarding the assessment of foreigners from different cultures.

Italy, located in the center of the Mediterranean Sea and close to the Balkans, represents from a geopolitical point of view, a natural gateway to Europe and which it has historically played this role for a long time.

Nevertheless in recent centuries, Italy has primarily originated emigration flows, while maintaining a homogeneously autochthonous internal population. These circumstances have changed radically over the past two decades, during which political and economic transformation globally, involving Eurasia and Africa—and also South America—has led to significant levels of immigration to Italy. Today Italy has a strong presence of foreign citizens in its own territory, both resident and transit to other European countries. This is a population with a specific age and gender constitution, according to different nations of origin, mostly consisting of workers seeking employment. Italy must now find a way to integrate both Italians and foreigners and migrants of different origins.

This presentation briefly describes quantitative and qualitative features of the foreign population living in Italy. The presentation also deals with this issue in terms of criminological survey, giving statistical information about the number and countries of origin of foreign people imprisoned in Italian jails.

According to Italian law, a judge or a lawyer can request a forensic psychiatric assessment of an offender, if there is reasonable suspicion that he/she is mentally ill. Usually the court asks the expert if the criminal is liable for his actions ("Imputabilità"), if he/she is a danger to society because of his mental disorder ("Pericolosità sociale"), and if he/she is able to participate in the criminal trial ("Capacità di stare in giudizio"). As the number of foreigners living in Italy has increased in recent years, there are currently a significant percentage of forensic psychiatric assessments being performed on criminals from very different cultures. This is new for the Italian forensic psychiatrists, who are facing new assessment difficulties and do not always have available the scientific instruments needed for a reliable cross-cultural assessment.

Case Report: This presentation is of a case concerning a double homicide committed by a foreign citizen residing in Italy as an example of the difficulty of the forensic psychiatric evaluation on persons from different cultures. The man came from sub-Saharan Africa, and had lived in Italy for about ten years before committing the crime. He killed his wife and another woman who lived with him with a sledgehammer, then went to his neighbors to report his crime. The genesis and dynamics of crime were influenced by religious and mystical beliefs which are maybe difficult to analyze and understand for a European citizen, but rather widespread and accepted in some rural areas of the country of origin of the offender. In order to answer to the judge's requests, the experts had to distinguish between physiologically different

beliefs merely due to the offender's different cultural context, and possible psychopathological convictions.

This presentation hijghlights the critical issues facing the forensic psychiatrist and points to the need to develop a model of cross-cultural approach to forensic evaluation of mental disease.

Conclusion: Italian training for psychiatrists and psychologists does not include compulsory academic courses in cross-cultural assessment. Migrants' anamnesis is mostly unknown, as it is very difficult to reach healthcare services in their country of origin. Versions of psychological tests validated for the countries of origin of the subjects to be examined are not always available. Intercultural mediators are few and are frequently not available for all migrants' ethnicities. Translation warps the content of the psychiatric interview.

Today in Italy, there are no guidelines for forensic psychiatric assessment of migrants. Experts' reliability depends on skills they have possibly acquired outside of their compulsory academic career. There is a need to develop national quality standards to ensure the individuals' equality of treatment under the law.

Foreign, Assessment, Criminality

I15 Violence and Epilepsy: The Importance of Prompt Identification of Post-Ictal Psychosis-Description of a Case

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After attending this presentation, participants will be able to recognize the features of the relationship between aggressive or violent behavior and epilepsy, with particular regard to the role of post-ictal psychosis.

This presentation will impact the forensic science community by providing understanding of the relationship between aggressive or violent behavior, epilepsy, and psychotic disorders with the aim of improving knowledge and prevention.

Aggressive behaviors occur in many different circumstances in society, and patients with epilepsy are not immune to being involved in aggressive acts. However, erroneous beliefs and prejudices linking epilepsy with violence have disproportionately emphasized the nature of this relationship. This notion acquired a highly stigmatizing value in the late 19th-century when the criminologist Cesare Lombroso promoted the association of epilepsy with aggressive sociopathic tendencies on the basis of degenerative theory. These distortions aggravate the psychosocial stigma already associated with epilepsy and have led to the questionable attribution of epileptic seizures in some cases of violent crimes or episodic aggressive outbursts.

Even today, the erroneous conviction that epileptic patients should be prone to violent actions and aggressive behavior represents a controversial subject especially in the field of forensic psychiatry.

While it is unclear that patients with epilepsy exhibit increased aggression, aggressive acts have been seen in association with seizures themselves. The prevalence of aggressive behavior in epilepsy has a rate that goes from 5% up to 50%. This significant variance depends on the different kind of sampling of patients.

Based on temporal relation of the crises, there are three different types of aggressive behavior: ictal aggressiveness, post-ictal aggressiveness, and inter-ictal aggressiveness. Most commonly, aggression may occur in the post-ictal state and can even be seen hours to days after initial periods of confusion. In particular, post-ictal violent behavior may be seen in association with Post-Ictal Psychosis (PIP).

PIP is characterized by a cluster of seizures, followed by a lucid interval, followed by the sudden eruption of a clinical disorder with a mixed affective picture, often accompanied by religious delusions and fear of impending death, lasting usually a matter of days. Episodes of PIP are comparatively common and could be dangerous, though (fortunately) are often treatable.

The following case presentation is representative of a violent act during an episode of PIP. A 29-year-old single Caucasian male was arrested and charged with attempted murders. He allegedly assaulted his mother and his brother with a knife. In the anamnesis, he had a 20-year history of paroxysmal episodes of "blanking out." At age 12, episodes of loss of consciousness began to follow these attacks. As he aged, the seizures increased in intensity as well as frequency, despite maximum drug therapy. At age 25, the first manifest post-ictal mental derangement occurred, after several bouts of untreated seizures. Thereafter, his post-ictal episodes recurred two times before the arrest, because he decided to stop the medication.

A few days before the crime, he experienced two grand mal seizures followed by the onset of multiple religious and persecutory delusions, thought broadcasting, feelings of being controlled by others, and command hallucinations. Because of a belief that the world was going to end, he left the house where he had been living and went to his parents' home. During the night, he woke up with a sensation of being persecuted by his mother and brother. He heard God and Satan arguing. God screamed, "No!" Satan bellowed, "They deserve to die." The two voices roared at each other, becoming one horrible overwhelming command, "Do it!" Fortunately, the intervention of neighbors saved the victims' lives. From a psycho-legal point of view, he was found not guilty on the grounds of insanity.

In light of the case presented, it is important to highlight that the assessment and management of violent behaviors in the patient with epilepsy requires careful consideration of several factors: whether the act is directly related to the epileptic seizure, a feature of post- or pre-ictal mental state changes, or a function of other conditions that increase the risk of aggressive behavior. Evaluation also requires appreciation of the patient's mental state and the social context in which the violent act occurs.

In the case of psychotic episodes, it is particularly crucial for the prompt identification of the relationship between the disorder and epilepsy, because patients should be treated differently according to the various pathophysiologic backgrounds, and because, in case of PIP, the most powerful and effective recipe for controlling the risk of violent behaviors is seizure reduction or elimination.

Epilepsy, Post-Ictal Psychosis, Violence

I16 Postmortem Prolactin in Suicides: Is it an Indicator of Antemortem Stress?

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After attending this presentation, the attendees will be able to recognize if postmortem prolactin levels are raised and possibly associated with antemortem stress in suicides.

This presentation will impact the forensic science community by developing an understanding of the possible trend and association of postmortem serum prolactin levels with antemortem stress and successful or completed suicides.

Stress is inevitable in today's life. A relationship between psychological stress and deliberate self-harm is well-established. Every year, over one million people commit suicide, and around 10 to 20 million non-fatal attempted suicides are reported worldwide. The World Health Organization estimates completed suicides as the 13th leading cause of death worldwide. Prolactin is a hormone secreted by lactotrope cells located in the anterior pituitary gland. It is mainly responsible for lactation, sexual arousal, myelination of neurons, surfactant synthesis in fetal lungs in humans, and is thought to play a significant role in the human stress response. The present research studies the postmortem plasma prolactin levels in completed suicides and tests the hypothesis that postmortem hyperprolactinemia is related to antemortem stress. This preliminary investigation is done to study the relationship between

completed suicides and serum prolactin, and to find if postmortem prolactin levels are raised in completed suicides.

The present prospective autopsy-based study for the biochemical estimation of serum prolactin was conducted at the Department of Forensic Medicine at Kasturba Medical College in Mangalore. An approval was received from the institutional ethical committee prior to conducting the study. Suicidal death among males during May and July 2010 were included in the study. Postmortem blood samples were collected from the right femoral vein before the start of autopsy and *in vitro* quantitative analysis of a non-hemolyzed blood sample was performed using electrochemiluminence. The normal range of serum prolactin in males (according to the chemiluminescence technology) ranges between 4.8ng/ml and 15.2ng/ml. All adult male autopsy cases of suicide with a survival period of less than 12 hours and postmortem interval of less than 24 hours were included in the study. Cases with other associated causes of hyperprolactinemia and hemolyzed blood samples were excluded from the analysis.

All the victims of suicide included in the study were males aged between 21 and 60 years. The mean age of the victims was 39.10±10 years. Most (90%) of the victims were married. Hanging was the preferred method of suicide (90%). Serum prolactin levels in cases of suicides ranged from 6.3ng/ml – 34.0ng/ml. The mean serum prolactin level among the cases was found to be 15.7±8.3ng/ml. A mean serum prolactin level of 14.97±8.3ng/ml was observed in the cases of suicidal hangings. The serum prolactin levels in cases of suicides were arbitrarily grouped into three categories: less than 10ng/ml, 10ng/ml to 15ng/ml, and more than 15ng/ml. It is observed that only 20% of the suicidal victims had a serum prolactin level < 10ng/ml, 40% had a prolactin level between 10ng/ml to 15ng/ml, and 40% had a serum prolactin levels > 15ng/ml.

The prolactin levels using postmortem blood samples in completed suicides were successfully determined. The mean postmortem serum prolactin level was found to be marginally higher in suicidal deaths, suggestive of a possible association between serum prolactin, stress, and suicides. The preliminary investigation is suggestive of a possible trend and an association of postmortem serum prolactin levels with antemortem stress and completed suicides. It is difficult to avoid stress, but suicide as the result of stress can be prevented by the early diagnosis of signs and symptoms of stress, with specific interventions toward preventing suicide. The association, however, is not strong and needs to be studied further in future studies.

Prolactin levels can be successfully determined using postmortem blood samples. This research emphasizes the importance of forensic pathologists in establishing causes of suicides at autopsy.

Suicide, Antemortem Stress, Prolactin

I17 Sidewalk Hermits: Homeless Perpetrators and Victims of Crime—Preliminary Results of an Italian Study

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After attending this presentation, attendees will better understand the dynamics regarding homeless perpetrators and victims of crime in Italy.

This presentation will impact the forensic science community by presenting some of the challenges that professionals in criminology and legal medicine face when dealing with crime among homeless people.

A homeless person may be defined as "a person in a state of tangible and intangible poverty, bearer of complex, dynamic, and multiform hardships."[1] The study presented here was conducted in collaboration with the Italian Railway Police from a criminological perspective, and focuses on homeless perpetrators and victims of crime. For this research, 47 cases have been studied and the socio-demographic variables included:

Age: The majority of subjects were male (72%) and 28% were female. The average age of the subjects observed was 49.8 years. The average age for males was 48.4 years and 53.3 years for females.

Nationality: 74% were Italian; 26% were foreign (most of whom were Northern and Eastern Europeans).

Education: 47% had five years of schooling; 19% had eight years of schooling; 6% had 13 years of schooling; and in one case, 18 years of schooling. Almost 30% of the subjects had no schooling at all.

Duration of Homelessness: 40% were homeless for more than five years; 47% were homeless between one and five years; and 13% were homeless for six months.

Events Leading to Homelessness: Loss of employment (26%); departure from home (20%); immigration (10%); divorce (10%); home eviction (8%); death of a family member who was the only source of income (4%); financial failure (4%). The literature shows that divorce and poverty, as well as family and living problems, predispose women to homelessness and to being victimized by crime. The literature also notes the role that mental illness and substance abuse play. Drug and alcohol use are predisposing factors for homelessness, the commission of crimes, and being the victim of crime.[2] In addition, most mental disorders diagnosed in the homeless are correlated to substance abuse, followed by mood, psychotic, and anxiety disorders; anti-social and personality disorders; and dual diagnosis.[3-6]

Criminal Aspects of Sample: Fifty-one percent of the crimes committed generally involved offences against property or violence perpetrated against another person. This is in agreement with the literature, which reports that such crimes among the homeless are tied to the acquisition of, and selling of, illegal substances.[7] Another interesting fact taken from the literature regarding the homeless is the high percentage of crimes committed against women. This has also been confirmed by the sample. Forty-three percent of the subjects studied had no previous contact with the legal system, neither as perpetrator, nor as victim.[8] Only 20% had been previously incarcerated. This datum differs from the literature a bit, which reports higher percentages of incarceration of homeless people with respect to this sample. The relationship between homelessness and incarceration is reported in various studies: 73% of males and 27% of females had been arrested at least one time.[9] Gardiner and Cairns (2002) reported that 77% of male subjects in their study had been previously arrested.[10] Moreover, being homeless increases the chances of being detained by the police after being stopped by them. And finally, physical and sexual violence constitutes another significant risk factor that may lead to homelessness.[11] In the end, physical and sexual violence is a significant risk factor for becoming homeless.

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Homeless, Crime Victims, Perpetrators

I18 Is There Such a Thing as Internet-Mediated Homicide?

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After attending this presentation, attendees will reflect on a specific type of murder related to the use of the internet, chat lines, and chat rooms.

This presentation will impact the forensic science community by assessing the role of the increasing use of the internet in the etiology of sexual crime or homicide.

In the literature, the terms “internet homicide,” “internet chat-room killer,” and “Craig’s List killer” have been coined to indicate the scenario in which a victim of homicide is met through a chat line or chat room and lured to death at the hands of the murderer. Various criticisms have been made of this new concept, on the grounds that the outcome is no different from that of other homicides committed without the use of web resources, and so the method used has no particular influence. Indeed, it has been claimed that informatic crime just reflects a technological change in the nature of crime rather than a new form of criminal behavior attributable to the use of the internet for criminal purposes.

This scenario offers points for reflection on the nature of web-mediated victim-murderer interactions, to assess the effects on the planning and commission of the crime.

These reflections were prompted by experience as expert forensic psychiatry witnesses in a case in which the murderer had confessed to having had an exclusively virtual relationship with the victim lasting about four months, in which they spent up to 14 hours a day on a chat line. Their relationship was characterized by a strong emotional involvement of the man, who left the girlfriend he was living with because he claimed to be in love with the woman he had met on the web. Importantly, the murderer and his victim had some “online sex” experiences, but he quickly started to show conflictuality and instability motivated by jealousy, which put the woman off. After threatening to commit suicide “live” on the web (brandishing a gun – perhaps a toy) to prevent the woman from ending their virtual meetings, the man went to her home and, during their first and only real meeting, he killed her with a blunt instrument he had carried with him.

The recorded dialog between the aggressor and victim (Murderer: *“How could you do this to me?”* Victim: *“Bastard, get out! I’ll fuck with whoever I want!”*) reveals the typical victim-murderer relationship but, although this had already been expressed in a virtual reality scenario, it had never before been translated to the real world.

The man had previous convictions for drug peddling and for attempted murder of his ex-wife discovered in the act of adultery, and violence against her companion.

The case appears to be an example of the destructive potential of passage from a virtual reality to the real world. It illustrates the risks of losing both a sense of reality and a sense of limits, making it difficult to separate what is real from what is imaginary and has never been real, with all the negative consequences that can stem from this loss of the sense of reality.

The woman’s desire to end an exclusively virtual relationship was seen by the man as a real, traumatic experience. It injured his deepest emotions and his anger, symptomatic of the narcissistic wound he felt he had received, can be classified as “one of the most pernicious afflictions of the human psyche,” because “if the man who feels anger is irrational, the man who feels hate is not, regardless of whether, in the given circumstances, the hate has a rational foundation or not.”

All this demonstrates that it is possible to claim that there really is such a thing as Internet-correlated homicide, because in this case the quality and quantity of the Internet interactions progressively altered the man’s perception of the real relationship between himself and the victim. This culminated, in their first and only meeting, in murder provoked by her rejection; the pain he suffered was undoubtedly real, and all the more incomprehensible and deep to him because of the pleasure he had derived from their virtual encounters.

Virtual Identity, Sexual Crime, Homicide

I19 Psychopathy and the Cinema: Fact or Fiction?

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After attending this presentation, attendees will gain knowledge about psychopathy and about the heterogeneity of this complex syndrome through movies and cinema history.

This presentation will impact the forensic science community, especially the forensic behavioral science community, by demonstrating the utilization of fictional characters as “teaching movies” for future generations of forensic psychologists and forensic psychiatrists.

This research investigated the relationship between cinema and psychopathy. They described and analyzed the portrayal of fictional psychopathic characters in popular films and over cinematic history.

Rather than assessing their commercial success or “aesthetic efficiency” and appeal, the the degree to which the portrayal was realistic from the clinical and psychopathological viewpoint of forensic psychiatrists, forensic clinical psychologists, and mental health professionals in general was assessed. Furthermore, by focusing on psychopathy in these fictional characters, the discussion of the portrayal of mental illness in cinema and the proposal of a fictional psychopathic character nosography for film history is possible.

The following international databases and film sources were used: “The American Film Institute (AFI),” “Academy Awards,” “Archive.org,” “Base de Données Françaises du Cinéma sur Internet (BDFCI),” “British

Film Institute (BFI)," "Cinebaseinternational," "Cinefiches.com," "Cinemovies.fr," "Cinoche," "CITWF," "Les Gens du Cinéma," "Greatest Films," "Il était une fois le cinema," "Internet Movie Database (IMDb)," "Oh My Gore!," and "Western Décrypté." All of these film databases were accessible on the internet without requiring authorization.

Based on these databases from 1915 to 2010, 400 films and the fictional, psychopathic-like profile characters therein were visualized and analyzed by senior forensic psychiatrits, forensic psychologists, and movie critics. As much information as possible was obtained about each fictional character, relying primarily on the films but also using any other available sources or documentation to make an accurate psychiatric diagnosis, specifically in terms of psychopathy. Because of the heterogeneous and abstract nature of these materials, neither "classical clinical evaluation" or psychometric tests were able to be performed. That is, the diagnosis and classification were the sole results of reviewing films and any additional information on a specific character and the discussions regarding how the character evolved in a specific context (e.g., interactions with others, personal history).

In a final step, the authors proposed a clinical fictional psychopathic nosography was proposed based on two documented psychopathic classifications: (1) Primary/secondary; and, (2) Classic/macho/manipulative/pseudopsychopath. From 400 films, 126 fictional psychopathic characters (21 female and 105 male) were selected based on the realism and clinical accuracy of their profiles. Secondary (71, 43%) and manipulative (47, 62%) subtypes were the most common in the female group, while secondary (51, 43%) and prototypical (34, 28%) were the most common in the male group.

Corresponding to the increased understanding of clinical psychopathy by professional mental health providers over time, the clinical description of and epidemiological data on fictional psychopaths in popular films have become more realistic. Realistic fictional psychopathic characters do exist, but they are in the minority. Despite this, they continue to contrast with their very interesting clinical descriptions, especially since 2000. These characters, which mirror some types of our society, are very important for the cinema itself and art in general, but mostly for future generations of forensic psychologists and psychiatrists as pedagogic materials.

Psychopathy, Forensic Psychiatry, Cinema

I20 Unusual Innovative Perspectives of Research on Human Violent Behavior

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After attending this presentation, attendees will be informed about the rising need to change research approach in order to deepen insight on the possible genetic bases of human violent behavior.

This presentation will impact the forensic science community by reviewing the results of most important genetic studies on violent behavior, and emphasizing the importance of the interdisciplinary scientific cooperation.

The link between genes and aggressiveness and its inheritability has been one of most debated issues in the criminologic arena, and perhaps is still a controversial topic. Among several theories on violent behavior, Darwin's Natural Selection theory, Lombroso's Intuitions, the Terrie Moffitt's developmental theory of crime, and the General Aggression Model are considered of major interest.[1-5]

Common sense questions need a proper answer: Do we all feel the same instinct to quarrel, punch, or kill? Do we observe the same aggressiveness in primary school children that punch over a trifling argument or in two males brawling, or punching, or even killing, while competing for the same female? And what about conflicts between husband and wife, or mother and son? Do we note the same kind and intensity of aggressiveness in a man resolute to rob a bank, but rapidly prone to murder the bank clerk, even before the policeman rushes to the crime scene after the alarm signal? Or in an adult subject who rapes a child; in a woman killing herself, or in twins both committing similar violent crimes?

Even if it is generally established that environmental, familiarial, educational, and cultural inputs influence the behavioral development, it's not enough of an explanation from a scientific point of view. More probable, it is: *individual genetic background* determining a sort of predisposition in aggressor habitus either in victimal status; *the circumstances* under which the violent act took place; and, *the momentary chance,* interacting together. How? This is the main question. The hypothesized existence of "warrior genes" has not ever been supported by scientific evidence. Notwithstanding, there are many investigations suggesting that genetic factors, such as hormones, neurotransmitters, enzymes, and endophenotypes have a leading role in human violent behavior.[6-10] The increasing importance of genetic factors in aggressiveness can't be undervalued any longer, and should be considered in a more complex perspective together with "the circumstances" and "the chance."

Consequently, an innovative study approach on this topic should be developed, providing a multitask research unit composed of more traditional specialties (psychiatrists, psychologists, sociologists, criminologists, and medical examiners) and also by innovative, maybe unforeseen, figures such as the anthropologist, the ethologist, the expert in non-verbal communication, and the genetic biologist. The purposed multitask teamwork should be proposed and applied with the same research program in many different countries, to share and compare the obtained findings, in order to understand any further aspect of the human violent behavior.

A brief review of the research program will be addressed with attendees.

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Human Aggressiveness, Violent Behavior, Research Unit

I21 Clinical and Treatment Reality in Italian Female Perpetrators of Crimes Considered "A Danger to Society"

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After attending this presentation, attendees will understand the importance of the real incidence of psychopathy in women for the planned treatment of new inmates.

This presentation will impact the forensic science community by assessing the rates of diagnosis of psychopathy in women affected by a serious mental disorder and considered at high risk of criminal reiteration.

The incidence of psychopathy is estimated to be 0.5% – 1% of the general population, while as many as 20% – 25% of prison populations qualify for the diagnosis.[1] The construct of psychopathy is essential in explaining criminal behavior, but unfortunately, empirical research on psychopathy in women has been inconsistent. In selected populations with a higher frequency of behavioral problems and higher rates of criminal behavior, men more often qualify for the diagnosis than women. Grann found that only 11% of female violent subjects met the criteria for psychopathy, as opposed to 31% of male violent subjects. In prison populations, other studies found rates of 16%[2] and 17.4%[3] among women, substantially lower than the rates for men in prison.

Furthermore, although men and women share most interpersonal and affective traits, as well as psychopathic behaviors, they may rely on different tactics to achieve the same goals. Considering these differences, the increase in female criminality demonstrates the need to gain a better understanding of the construct of the disorder in women in order to validate results already obtained in large samples and to develop suitable, objective evaluation tools for making reliable predictions of re-offenders, institutional facilities needs, and treatment responses.

In Italy, all women perpetrators of a crime who are not considered penally liable because they were affected by a serious mental disorder at the time of the crime, and considered at high risk of criminal reiteration (a danger to society), are hospitalized in the "Ospedale Psichiatrico Giudiziario" (OPG) (Judiciary Psychiatric Hospital) of Castiglione dello Stiviere, in northern Italy. This facility also admits men with the same characteristics. A recent research conducted in this hospital facility, using the PCL-R,[4] demonstrated a 30.7% rate of psychopathy among the inmates affected by a personality disorder, (16.9% male and 13.8% female); the mean score at the PCL-R in the sample was 28.5 for women and 26.5 for men. The research also showed that in 85% of males with a high psychopathy score, the admission diagnosis at the OPG was antisocial behavior, whereas in women a diagnosis of a borderline disorder was more common. These results are in line with those in the recent international literature that point out not only the different phenotypical manifestations of psychopathy in the two sexes, but also the likely general underestimation during risk assessment, of the violent potential and rates of female psychopathy.[5] This is also because few data are yet available on this issue.

In the light of these considerations, an observational study of the entire female population hospitalized in the OPG of Castiglione delle Stiviere was conducted to individuate the real incidence of psychopathy. In fact, it is very probable that the rates of diagnosis of psychopathy in women are too low. If this hypothesis is confirmed, admissions to the OPG failing to make a correct diagnosis of so important an element as psychopathological traits may have a negative impact on the planned treatment of new inmates.

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Psychopathy, Social Dangerousness, Treatment

I22 Forensic Psychological Issues From Terrorism to Officer-Involved Shootings: U.S. Border Patrol Critical Incident Investigative Teams

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The goals of this presentation are to: (1) explore the forensic psychological factors associated with traumatic incident management, debriefing, substance abuse, self-assessment, and suicide prevention for the Border Patrol CIIT; (2) explore the culture of the Border Patrol; and, (3) fuel a foundation for additional research.

This presentation will impact the forensic science community by providing professional dialogue that will result in best practices in this area.

The federal government maintains hundreds of ports of entry. The length of the United States – Canada and United States – Mexico borders makes them convenient targets for illegal border activity. The responsibility of the U.S. Border Patrol is to no surprise directed at preventing Homeland Security breaches due to dangerous people and potentially dangerous situations. For example, following the terrorist attacks of 9/11, U.S. Homeland Security, and specifically the Border Patrol, became increasingly sensitive to border protection policy. There was intense discussion and considerable disagreement regarding immigration from the border with Mexico that added to the pre-existing concerns about drug trafficking and other border crimes. Also fueling tensions were competing economic needs reflected in Mexico's job demands and the need for a cheap work force. For example, the hourly wage in Mexico in 2000 was less than two U.S. dollars. By contrast, the earnings for unskilled undocumented workers in the U.S. are almost three times higher. Monies returned to Mexico also impacts their economy.

The USA Patriot Act was a major piece of legislation aimed at addressing the need to protect Americans from perceived threats posed by terrorists. To address those concerns, the number of Border Patrol agents increased from 3,965 in September 1993 to 12,349 in September 2006. In the fiscal year 2009, there were about 20,000 Border Patrol agents. The Border Patrol mission obviously applies not only directly to terrorists but terrorist weapons. Among the objectives of the Border Patrol is the planned use of information, integration, and rapid response that is crafted to protect the borders of the United States. The Border Patrol objectives of managing risks, increasing community engagement, and strengthening an investment in people all have underlying forensic psychological elements. The work of Border Patrol agents is hazardous and involves a significant amount of stress. For example, four Mexican nationals were charged in the shooting death of a U.S. Border Patrol agent. This incident was believed to be connected to the controversial "Fast and Furious" gun-smuggling operation. Border Patrol agents are also subjected to accusations of corruption and abuse of migrants. The Border Patrol's border enforcement has the doctrine of "prevention through deterrence" which also had implications for this unit. For example, the border stations San Diego, El Centro, Nogales, and El Paso have seen the construction of fences, lighting, and an increase in agents. Yet, some remote areas (e.g., mountains and deserts) were less

protected and resulted in more risky attempts by migrants. The U.S. Border Patrol has a unit referred to as the "Critical Incident Investigative Team" (CIIT). The CIIT has witnessed an increase in the number of migrant deaths due to dehydration, hypothermia, and being double-crossed by coyotes hired to smuggle them across the border.

In many respects, CIIT functions as an investigative nexus to address a wide range of incident issues that arise as Border Patrol agents carry out their enforcement duties. Yet, there are unique sources of tension (e.g., supervisor issues, suicidal behavior of Border Patrol agents, and Latino law enforcement cultural factors) in the U.S. Border Patrol. The first learning objective of this presentation explores the forensic psychological factors associated with traumatic incident management, debriefing, substance abuse, self-assessment, and suicide prevention for the Border Patrol CIIT. The second learning objective explores the culture of the Border Patrol. The third learning objective is designed to create a theoretical foundation for additional research. The community is expected to profit from a professional dialogue that will result in best practices in this area.

Forensic Psychology, Border Patrol, Critical Incident

I23 Testing Police Suicide Notes: Do General Population Notes and Police Testing Notes Significantly Differ?

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After attending this presentation, attendees will learn about police suicide reporting rates. Attendees will learn how the software tool SNARE provides suicide note authentication using both text-analytic, quantitative classification, and qualitative assessment based on database extraction. Attendees will learn how this method has been used to determine if police suicide notes are or are not significantly different from general population suicide notes. Attendees will also learn how validation testing is performed using behavioral and linguistic datasets, including highly sensitive data, and human subject protections for survivors of suicide.

This presentation will impact the forensic science community by showing how suicide is an international issue that affects American policing at a rate substantially higher than the general American public and only slightly lower than the American military. In the police culture, suicide is considered very shameful, which may cause an under-reporting of police suicide. Yet police officers enter their profession having met stringent standards for physical condition, mental health, and problem-solving ability, including interpersonal skill. This paper presents an empirical study of police suicide notes to determine if police suicide notes are or are not significantly different from general population notes. The method presented here, using both a computational tool and qualitative assessment, illustrates the particular human subjects protection required for validation testing using behavioral and linguistic datasets, and thus also advances the methodology of forensic science.

More than almost any other profession in the United States, policing screens its new hires for physical condition, psychological health, and problem-solving ability, including optimal interpersonal skills. Police officers begin their careers with great psychological strengths.[1] Yet it cannot be disputed that suicide occurs among police officers. Even our best can and do succumb to suicide. Miller states that "more police officers die by their own hand than are killed in the line of duty".[2]

Suicide risk is clearly related to occupational hazards of the policing profession. Such hazards include atypical schedules disruptive of circadian rhythms and long work hours, participation in violent events, constant dealings with difficult people, and prevalence of weapons.[3-8] These hazards, over time, can lead to depression, divorce or family isolation, and post-traumatic stress disorder, all of which are potential triggers for suicide.

These occupational hazards, especially the association with violent, traumatic events, help make sense of recent scholarship on the rate of police suicide. Only recently have national, empirical studies to determine police suicide rate been initiated through The Badge of Life Program.[9] Two facts can be gleaned from these studies. First, the rate of police suicide is higher than the general population. According to O'Hara and Violanti (2009), the suicide rate for police officers was 17/100,000, compared to the rate for the general public of 11/100,000 and 20/100,000 for the Army.[10] Loo had also found higher suicide rates in police than comparison populations.[11]

Second, the rate of police suicide is controversial because there may be under-reporting of police suicide. Just as the shame associated with rape in the American culture may cause actual rapes to go unreported, the shame associated with suicide in the police culture may cause actual suicides to be classified as other types of events or "undetermined." Violanti demonstrates that "male police officer deaths had a 17% increased risk of being misclassified as undetermined" than both firefighter and military occupations, and the risk of misclassification was far higher for female and African American police officers.[12]

In this context, the question is posed: are police suicide notes significantly different from general population suicide notes? This hypothesis was tested using SNARE, a suicide note assessment research tool for identifying, classifying and assessing suicide notes. SNARE combines a computational, quantitative tool and a database extraction feature for qualitative assessment. The quantitative tool in SNARE currently has obtained an accuracy rate of 80% on a dataset of 400+ real suicide notes and 500 control documents. When the real suicide note data is limited to brief notes (45 words or less), the accuracy rate increases to 86%. The statistical classifier is a linear discriminant function analysis using leave-one-out cross-validation. The reported accuracy rates are the average of true positives for the real suicide note class and the control document class. Preliminary results using only the quantitative component of SNARE have not shown differences between police and general population suicide notes.

Finally, forensic linguistic data safeguards are considered. While all suicide note research must take into account human subjects protections (44 CFR 45), research into police (as well as military) suicide must be especially cautious in collecting data, securing the data, and protecting survivors' privacy. Discussion includes how each of these issues is handled so research partnerships could be established between institutions.

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Suicide Notes, Validation Testing, Human Subjects Protections

I24 Veterans' Courts: Trend for the Future?

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After attending this presentation, attendees will be able to describe the purpose and functioning of the Veterans' Court. Attendees will understand the history of attempts to address mental health issues in veterans who are entangled with the criminal justice system and will be brought up to date on current data on effectiveness of the Veterans' Court.

This presentation will impact the forensic science community by updating attendees on an important current trend in tending to the needs of veterans in the criminal justice system, a population which has vastly expanded since the Iraq and Afghanistan conflicts.

Since the inclusion of Post-Traumatic Stress Disorder (PTSD) in the DSMIII in 1980, the legal system has recognized the potential value of PTSD in shielding veterans from the criminal justice system. However, even before then, the courts had considered defendants' war experiences to assist in weighing criminal responsibility and appropriate punishment.[1] The first wave of veterans to employ the PTSD defense were Vietnam Veterans who received an often-negative response to their claims of legal insanity due to combat related PTSD.[2] With the end of the Iraq war in 2011 and the anticipated return home of most troops from the Afghanistan conflict by the end of 2014, the American mental health system is expected to be flooded with over 1.5 million veterans, with up to 35% suffering from PTSD.[3] There has been some evidence that violent crime rates are elevated among veterans returning from the Iraq war, and that violent crime is elevated among combat veterans in general.[4-5] As opposed to Vietnam Veterans, public attitudes toward Iraq/Afghanistan Veterans remain positive.[6] Thus, there is considerable interest in the utility of PTSD to help shield these veterans from the consequences of criminal behavior or to form the basis of treatment. PTSD may also be employed in arguments to reduce criminal responsibility.[7] Recently, a movement of Veterans' Courts which began in Buffalo in 2008, has expanded around the country.[8,9] In some districts, veterans convicted of felonies are eligible for services and counseling.[10] Veterans' Courts have sprung up to rectify the perceived injustice of punishing veterans for behaviors related to the symptoms of PTSD.[11] However, to date, there is little published data on the effectiveness of Veterans' Courts. Proponents argue that "treatment not punishment" both provides justice for veterans and helps reduce recidivism. However, others are troubled by the creation of a privileged class of criminal defendants. The paper presents the recent history of attempts to address the issue of veterans in the criminal justice system, the inception of Veterans' Courts, and the data available regarding effectiveness. The paper discusses the inconclusive nature of the evidence at present and suggests direction for future studies.

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Veterans, Treatment Court, PTSD

I25 Early Intervention Programs to Prevent Serious Juvenile Delinquency

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After attending the presentation, attendees will be exposed to research-tested methods for reducing juvenile delinquency recidivism and, more importantly, to understand research supporting interventions for early prevention of this delinquency.

This presentation will impact the forensic science community by educating attendees on the status of the scientific research in the prevention of delinquency.

This presentation will discuss the prevailing failed attempts at preventing juvenile delinquency and its recidivism. The predominant method for dealing with juvenile delinquents in the last three decades has been to treat many as adults with harsh sentencing.

Recognizing the developmental aspect of juveniles as distinct from adults, the first juvenile justice system was established in 1899 in Illinois and led to the creation of the first child and adolescent psychiatry clinic in 1909 in Chicago. This clinic was specifically created to aid the newly formed family courts in their adjudication of "wayward youth." Eventually all 50 states adopted these special courts to handle juvenile delinquency outside of the adult criminal courts. The goal of these courts was to help the youth return to a healthy path of development. Hence, there was no sentencing, only adjudication of the youth "for their betterment." Until *In re Gault*, children and adolescents in family court had few of the safeguards afforded to adults charged with crimes. Since the 1970s, with the dramatic rise in violent crime committed by adolescents, many juveniles have been returned to the jurisdiction of adult criminal courts. Society was outraged to see juveniles murder and rape and be released from custody at the age of 21 years, and further to have the juvenile records sealed. In some jurisdictions, the juvenile may begin in family court and be waived up to adult criminal court. In other states, such as New York, adolescents who commit specific crimes of violence or with a weapon are immediately sent directly to adult criminal court. Society has sought to reduce juvenile crime with longer incarceration with only modest success. Unfortunately, incarcerated juveniles, when released have been fully educated to be better criminals. Spending time with more experienced criminals provides an unwanted fertile environment for teaching adolescents exactly what we do not want them to learn.

In the last three decades there has been ample research to demonstrate that instituting multisystemic therapy for serious juvenile offenders, keeping them in the community with intensive intervention, can significantly reduce recidivism. When there is recidivism, it is less severe than in released incarcerated juveniles. Multisystemic therapy provides 24-hour available parental guidance, family therapy, individual therapy, group therapy, educational support, and quite importantly, a change of peer group. In New York City, there is the new mandate through the Juvenile Justice Initiative to implement interventions to keep juvenile offenders in the community rather than sending them to be incarcerated.

However, let's look at how teaching prosocial values in early childhood can reduce the incidence of first-time juvenile delinquency. Programs such as the Perry School Project will be discussed to demonstrate that although somewhat expensive, these innovative programs nonetheless are quite cost-effective as the cost to society of adjudication, incarceration, and victim damages are significantly greater. Along with teaching prosocial values, there has been renewed interest in early identification of youth at risk for developing Antisocial Personality Disorder. An update will be given on the status of both promising approaches in early intervention to prevent serious juvenile delinquency and hence adult criminality.

Juvenile Delinquency, Prevention, Early Intervention

I26 Anti-Bullying Legislation: Have the Laws Gone Too Far or Are They Still Insufficient to Combat Bullying?

Karen B. Rosenbaum, MD, 200 W 85th St, Apt 1B, New York, NY 10024*

After attending this presentation, attendees will understand the impact of bullying on children, schools, and the community. Attendees will understand the need for anti-bullying legislation and the issues that have risen since some of the laws have been implemented.

This presentation will impact the forensic science community by providing an update of anti-bullying legislation and placing it into a clinical and forensic context.

Over the past several years, with the increase in teenage suicides, social networking, and community awareness, bullying has become recognized as a community health problem as well as a political issue. Over the past three years, there have been many high-profile cases of teenage suicides precipitated by bullying, which then prompted state laws to change. Phoebe Prince, a 15-year-old Irish immigrant, killed herself in South Hadley, MA, on January 14, 2010, after being bullied by several students in her high school. On September 22, 2010, Tyler Clementi, a Rutger's freshman, jumped off the George Washington Bridge after being outed over the internet by his roommate. Jamey Rodemeyer committed suicide in Buffalo in September 2011 after being tormented by cyberbullies.

These and many other tragic cases influenced a change in the law regarding bullying. In response to Tyler Clementi's death, New Jersey passed an "anti-bullying bill of rights." Since 2002, New Jersey had an adequate anti-bullying law in which some school districts complied and others did not. The new bill, passed in 2010, which went into effect in September of 2011, made it clear that responding to threats of bullying and intimidation was not optional. The problem with the New Jersey law and several other new state laws regarding bullying is that they did not provide the funding for schools to uphold them. In January of 2012, the law was declared unconstitutional because it was an "unfunded mandate" that funneled resources away from other programs. The law was well intentioned but did not provide the resources necessary to implement the programs mandated in the law.

In a recent article in *The Wall Street Journal* called, "Stop Panicking About Bullies," Mr. Gilespie questions whether or not America is "really in the midst of a "bullying crisis."[1] He does not believe that childhood bullying is on the rise. He also feels that the laws designed to prevent bullying are "likely to lump together minor slights with major offenses." He feels that children today are tamer and less mean than children growing up in the 1970s and 1980s. While there may be some truth to this, children growing up in the 1970s and 1980s did not have access to email, social networking sites such as Facebook and Twitter, or mobile phones. Children who were bullied thirty years ago were most likely only bullied during school hours and home served as a somewhat safe haven. Today, children can be potentially exposed to bullying every time they turn on their computer or smart phone.

After high-profile cases such as the deaths of Phoebe Prince and Tyler Clementi, there have been new state legislations mandating schools to have formal policies against bullying. This presentation will address some of the new laws designed to prevent bullying in schools, and the issues that have arisen since these laws have been in place.

Reference:

1. Gillespie N, Stop Panicking About Bullies, *The Wall Street Journal*. March 31, 2012, Page C1.

Bullying, Legislation, Schools

I27 Bullying Victimization in Adolescence: A Precursor to Future Delinquency?

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The goals of this presentation are to: (1) understand the relationship between bully victimization in adolescence and future delinquency; (2) identify risk factors that may predispose these victims to becoming delinquent; and, (3) discuss the various types of delinquent acts that may be committed.

This presentation will impact the forensic science community by better recognizing the victims of bullying who may be at risk for developing delinquency, so that early, appropriate intervention can be implemented to deter these victims from a delinquent path.

Bullying is defined generally as a specific type of aggression which includes the following: the behavior is intended to harm or disturb; the behavior occurs repeatedly over time; and, there is an imbalance of power, with a more powerful person or group attacking a less powerful one. National surveys conducted from 2005 to 2009 indicate that up to 28% of youth, primarily during adolescence, reported having been bullied during the survey periods. It is known that perpetrators of bullying, or "the bullies," pose a significant safety risk to their fellow peers and others. In one National Institute of Child Health and Human Development survey, bullies were seven times more likely to report they carried a weapon to school in the prior month. Other studies have shown that bullies, identified by age eight are six times more likely to be convicted of a crime by age 24, and five times more likely to have serious criminal records by age 30.

Little information, however, exists about the victims of bullying and whether they engage in future delinquent behaviors. While research suggests that victims of bullying may experience serious emotional sequelae, including poor social-emotional adjustment, depression, psychotic symptoms, and even suicide, there are scant data regarding the victims' propensity for future delinquency. Does being bullied in adolescence, a critical time period for development, predispose one to engage in future delinquent behaviors?

Agnew's general strain theory endorses that adolescents who experience adverse circumstances are pressed into delinquency by negative emotional reactions, such as anger. Accepting that being a victim of bullying is an adverse circumstance, are there reasons, other than anger, that adolescent bullying victims engage in delinquent behaviors? That is, are there any identifiable risk factors that may predict the likelihood of delinquency for such individuals? Another issue to consider is the kind of delinquent conduct in which these victims may engage. In the recent past, media have shed light on school shootings, with many associating the occurrence of these acts with persons who have a history of bullying victimization. But not all victims of bullying use firearms against their peers. So what may lead a bullying victim to such extreme measures? It is important for the astute forensic practitioner to

remember that while a history of bullying is often present in such school shooting cases, most of these involve complex issues that are not simply limited to bullying victimization.

From a forensic psychiatric perspective, this presentation will review the literature to examine the aforementioned issues of whether bullying victimization in adolescence predisposes to future delinquency. In addition, it will identify potential risk factors for such a predisposition, as well as explore the various types of delinquent acts in which these victims may engage.

Bullying, Victimization, Delinquency

I28 Evolution of the Adolescent Brain: Science, Sentencing, and the United States Supreme Court

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After attending this presentation, attendees will be able to: (1) compare and contrast the outcomes of three recent landmark juvenile justice cases from the United States Supreme Court; (2) explain the scientific basis, reasoning, and principles which informed these opinions; and, (3) briefly describe how one state (Arkansas) is mobilizing to respond to the most recent of these landmark cases.

This presentation will impact the forensic science community by increasing awareness of how advances in the understanding of adolescent brain development are impacting juvenile justice sentencing guidelines.

The United States Supreme Court has had an active decade in the matters of juvenile justice. In *Roper v. Simmons* (2005), the Court found that the Eighth and Fourteenth Amendments forbid imposition of the death penalty on offenders who were under the age of 18 when their crimes were committed. In *Graham v. Florida* (2010), the Court held that, for non-homicide crimes, the Eighth Amendment does not permit a juvenile offender to be sentenced to life in prison without parole. And, most recently, in two cases (*Evan Miller v. Alabama* and *Kuntrell Jackson v. Ray Hobbs, Director, Arkansas Department of Correction*) which were decided together on June 25, 2012, the Court decided that the Eighth Amendment forbids sentencing schemes which mandate life in prison without possibility of parole for juvenile homicide offenders.

These three opinions have direct implications on state sentencing guidelines for juvenile offenders. This presentation will focus on the Court's use of science in formulating these opinions, and the role of factors such as an adolescent's maturity, development of sense of responsibility, vulnerability to negative influences and outside pressures, control over environment, and fixedness of character traits. As these factors can serve to inform the forensic evaluation of adolescent culpability, this presentation will include discussion through the lens of the 2011 Presidential Address at the Annual Meeting of the American Academy of Psychiatry and the Law, as well as "the evolving standards of decency that mark the progress of a maturing society" (*Trop v. Dulles* as cited in *Miller* and *Jackson*) in the context of contemporary psychiatry's bio-psycho-social framework.

Both the *Miller* and *Jackson* cases involved 14-year-olds convicted of murder and sentenced to mandatory terms of life imprisonment without the possibility of parole. Arkansas charged Jackson as an adult, and Miller was initially charged as a juvenile but his case was waived up to adult court. Discussion will include the implications of which court adolescents are tried in, as this also may hinge on the forensic evaluation of adolescent culpability.

Lastly, as the authors are faculty at the University of Arkansas School of Law, the presentation will include an opportunity for the audience to learn about how one state (Arkansas) is mobilizing to respond to the *Miller* and *Jackson* rulings, in terms of juvenile homicide offenders in Arkansas currently serving what until recently were mandatory life sentences without parole. As life-without-parole sentences are thought to "share some characteristics with death sentences that are shared by no other sentences" (*Graham*), these characteristics invite discourse when parole becomes a possibility.

Adolescent Development, Juvenile Justice, Sentencing Guideline

I29 Contemporary Neuroscience Affects Forensic Behavioral Science Standards

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The goal of this presentation is to incorporate the implications of important findings from neuroscience into the daily work of advancing and applying forensic behavioral science.

This presentation will impact the forensic science community by gaining appreciation of neuroscientific discoveries, delighting in the significance for forensic behavioral science in a variety of relevant social contests.

Examinations of forensic behavioral science in response to the February 2009 report of the National Academy of Sciences Report, *Strengthening Forensic Science in the United States: A Path Forward*, point to a gratifying array of established habits that currently promote a high level of quality in both training and practice. Although its advances already impact practice, training, and research in the field, surprisingly little has been said so far about the implications of neuroscience. Yet it is a rapidly proliferating area that can be expected to command more attention in the very near future.

Experiences so far have provided a basis for high expectations as neuroscientists continue making their discoveries. This trend began during the 1970s with the establishment in cats of a distinction between two kinds of aggression, affective and predatory, each associated with changes in the activity levels of different brain structures. During the same decade, the first pharmacological agent shown to lower aggressive behavior, lithium, was documented in humans. Soon after, there appeared several associations relating brain structures and behavior, including the amygdala and emotional memory, the prefrontal cortex and executive function, and the limbic system and impulsive reactions.

As we gain information about the functions of brain structures and their connections through the use of functional Magnetic Resonance Imaging (fMRI), we increasingly recognize serious risks of oversimplification. The human brain has 100 billion neurons each connecting to a rough average of 1,000 others, and there are several glial cells for each neuron. These cells are being found not so inert as was once thought. The amygdala itself is composed of several paired nuclei, making it more than a single organ. Moreover, concerns are now arising about the validity of once-established results in neuroscience.

Appropriate concerns are being raised about the use of neuroimaging in the courtroom. Images have an inherent convincing quality unlike galvanic skin responses and similar behavioral evidence. Valid information about the developing brain is likely to have a helpful place in the evaluation of competency to stand trial. At least since the trial of John Hinckley, Jr., we have seen controversies concerning the use of neuroscientific techniques to aid in the assessment of criminal responsibility. Fascinating results are emerging with respect to the detection of malingering and other lying. A recent civil case involved the use of fMRI to demonstrate a putative association between violent video game playing and violence in order to support the claim of the state that it had a compelling interest in regulating these videos.

The study of violent behavior stands to gain much if it undertakes to link the findings of neuroscience to the social contexts in which human brains give meaning to the making of choices, including the choice to behave violently.

Another area of growing concern is medicinal enhancement, the use of substances to bolster alertness, memory, cognition, and other functions, such as physical prowess. Some compounds are new; others, mostly psychotropics, are familiar but are being proposed for off-label uses. Forensic behavioral science has a role in assessing this practice, especially as it spreads to new social contexts. As

neuroscience continues to make its advances, it is increasingly important for forensic behavioral science experts to keep pace by applying close vigilant attention to the relationships among scientists working in different but interacting social contexts.

Neuroscience, Social Contexts, Practice Standards

I30 A Typology of Parricide Offenders

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After attending this presentation, attendees will be aware of: (1) four clinical types of parricide offenders; (2) the relationship of age to parricide offender type; and, (3) specific criteria required to make the parricide offender designation, criteria that are frequently associated with this type, but that are not considered essential to making the classification, and criteria that might be present.

This presentation will impact the forensic science community by identifying four types of individuals who kill parents in terms of social history, life circumstances, typical mental health diagnoses, and motivational dynamics related to the killings.

The killing of parents, increasingly referred to as parricide, has been documented worldwide. Although matricide and patricide are rare events, mental health professionals, the justice system, and the public alike look for reasons why sons and daughters kill parents. This presentation will discuss the characteristics of different parricide offender types based on research, the evaluation of parricide defendants, discussion of parricide cases consulted on, and the extensive knowledge of the literature. This presentation will list criteria *required* to make the parricide offender-type designation, criteria that are *frequently associated* with this type but that are not considered essential to making the classification, and the criteria that *might be* present. Three of these parricide types were introduced more than 20 years ago in the book, *Why Kids Kill Parents*, and have stood the test of time as referenced by other clinicians and scholars in the field. These three types are: the *severely abused child*, the *dangerously antisocial child*, and the *severely mentally ill child*. Among children, adolescents, and young adults, the severely abused child and the dangerously antisocial child are most common. Among older adult parricide offenders, the severely mentally ill and the dangerously antisocial types predominate. The fourth type, angry children with low frustration tolerance, is found among juvenile and adult parricide offenders.

- Severely Abused Children (SAC) kill their abusive parent to end years of abuse. They kill the abusive parent because they are terrified that they or other family members will be seriously harmed or killed. SAC are typically desperate and see no other way out but murder. These individuals typically have a longstanding history of depression and meet the diagnostic criteria for Post-Traumatic Stress Disorder (PTSD).
- Dangerously Antisocial Children (DAC) kill the parent to further their own goals. In these cases, the parent is an obstacle in their path to getting what they want. These individuals, for example, may kill to have more freedom, to continue dating a person to whom the parent objects, or to inherit money they believe is eventually coming to them. DAC have a pattern of violating the rights of others when it suits them. Depending on their age, these parricide offenders are often diagnosed as having a conduct disorder or an antisocial personality disorder. This type of parricide offender is far more dangerous to society than the first in terms of re-offending and hurting other people in the future.
- Severely Mentally Ill Children (SMIC) who kill parents typically have a longstanding history of mental illness. Diagnoses commonly made include psychosis and severe depression. SMIC are typically on psychotropic medication and are most apt to kill when they stop taking it.
- Angry children with low frustration tolerance appear to be raised by parents who do not enforce limits. The parents are often lax and might be overindulgent. As the children get older, they do not accept parental authority. As parents step in and attempt to set appropriate and needed boundaries, these children aggress because they have little frustration tolerance. Often their homicidal rage is fueled by alcohol and/or drugs.

Parricide, Matricide, Patricide

I31 Sociodemographic Data of Intimate Partner Homicide-Suicide Cases in Turkish Mass Media Between 2008 – 2011 Through Judicial Records

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After attending this presentation, attendees will learn about intimate partner homicide-suicide cases in Turkey between 2008 – 2011.

This presentation will impact the forensic science community by presenting information about intimate partner homicide-suicide cases in Turkey to point out some social and legal basis of problems.

The term “Homicide-Suicide” (HS) can be explained as “the committing of suicide by the same individual after he/she has commitment a homicide.” Some authors stated that the act can be defined as a homicide-suicide if the time period between homicide and suicide is less than 24 hours while other authors use a time period of a few days to one week. Other authors have used any time period in their definition. In order to evaluate the HS cases, different methods of classification are used by different authors according to psychopathological features of perpetrators, civil status of the relationship between victim and perpetrator, or the motivation of committing a homicide.

HS is most frequently seen among intimate partners and, within this scope, is an important part of sexual violence in society. Besides, violent features of these incidences mostly find a place in mass media. Thus, this study is planned to scientifically elicit the sociodemographic features of these mass media cases which have been constituting a good “image” of the general “picture” all around the country about Intimate Partner Homicide-Suicide (IPHS). Motivation of the perpetrator has also been included in the study as a sociodemographic factor. A media monitoring agency has been used with specific keywords to detect all the incidences of IPHS which had found a place in mass media between 2008 – 2011 in Turkey. The cases were then searched in the Turkish judicial system through the judicial archives of forensic medicine units, which are organized in the structure of the Council of Forensic Medicine, an unique official forensic medical expertise institution. The judicial records were evaluated in order to have the sociodemographic data of the victims and the perpetrators. The data collected were analyzed through descriptive statistics with SPSS 16.0 software, since it had been planned as a cross-sectional study.

As a result of the data analyses, 122 incidents of IPHS including 122 perpetrators and 157 victims in a time period of 48 months between January 1, 2008 and December 31, 2011 have been detected. Among victims, 35 were a third party instead of a spouse or intimate partner. Twenty-three among 35 were the mutual children of the couples constituted by the perpetrator and the victim. Ninety-seven point 5 percent of the IPHS perpetrators were male, showing that almost all of the sexual violence in mass media, in society, is against women instead of men. The mean age of the perpetrators was 41.54 (in the range 22 – 80 years) while the mean age of the victims was 35.56 (in the range 18 – 73 years). Also, 59.8% of the perpetrators and victims were found to be married officially during the incidences, while 52% were living separately during the incidences. The education level of 54.1% of the perpetrators were found to have a secondary school level of education. A psychiatric history was found in 7.4% (n=9) of the perpetrators, among

whom only two were found to have a psychiatric diagnosis. The fact that 19 perpetrators had been committing violence to the victim before the homicide incidence was determined among 22 perpetrators for whom data related to the violence commitment status were able to be obtained. Also, 56 (45.9%) perpetrators were found to have exhibited behaviors of murder contemplation, while in 87 cases (71.3%), the time interval between the homicide and suicide was less than one hour. In addition, 89.5% of the perpetrators committed suicide using firearms while 88.6% of the victims had been murdered by firearms, all of which can also be parameters to discuss the motivation as a power ratio between the victim and the perpetrator, a violent drive-control ability, and other psychiatric basis.

The limited data can be found to be related to the past sociodemographic features, especially of IPHSs. In order to be able to obtain more data and documents, judicial units' recording more data will provide more of a chance, especially for psychiatric, sociological and criminal researches. Even with these numbers, this study can be the most comprehensive case collection in Turkey related to IPHS cases in mass media, providing some significant awareness for evaluation and prevention of further cases.

Homicide-Suicide, Intimate Partner, Sociodemographic

I32 The Psychological Autopsy as Method in Case of Suicide by Hanging

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After attending this presentation, attendees will understand the role of the psychological autopsy in a case of suicide.

This presentation will impact the forensic science community by discussing the relation between a suicide case and the choice of suicide with psychosocial, environmental, and cultural risk factors.

Introduction: Suicide is a public health problem; as a general rule it is underestimated. Prevalence of death due to suicide is greater than cancer, respiratory disorders, and other common diseases. Every year, more than 10/100,000 people commit suicide, with an alarming rise in suicides among children and women 18 to 30 years old; specifically, it is a major preventable cause of death among children. Every country has a constant trend. Specifically in Italy, there is a low risk of suicide, with the northern regions exhibiting values almost double compared to the south. It's influenced by psychosocial, environmental, and cultural risk factors. There are many risk factors: advanced age, male sex, widowhood, divorce, previous attempts or intentions of suicide, depression, schizophrenia, drugs, unemployment, social isolation, suicide among relatives, hallucinations, and delusions of persecution. Suicidal ideation is based on both cognitive substrates and personality disorders along with interactions with family as impaired parent-child relationships, emotional dissatisfaction, self-harming behavior, frustration, social marginalization experience, and inability to recognize other's complex emotional states. Even families dominated by violence and abuse can generate potential candidates for suicide. Also, there is a clear association with groups, as with psychiatric patients and persons with several mental and physical illness. In particular, adolescents with intellectual disabilities often diagnosed with comorbid psychiatric disorders are a vulnerable population who may be at risk for developing suicidal thoughts and behavior. Many different factors may influence personal decisions about the choice of suicide modality of death. There is a clear indication of restricted access to lethal means associated with a decline in suicide, especially for methods with a high fatality rate. Suicidal setting analysis is performed by forensic pathologists with "psychological autopsy." This survey includes demographics, lifestyles, personality traits, personal and psychological data of suicide's victims, and suicidal reasons. The psychological autopsy is one of the most valuable tools for research into the suicide's death.

The goals of this study are to evaluate the psychological autopsy to understand reasons and origin of suicide cases.

Case Report: This case has been tested, through the method of psychological autopsy, the case of a 17-year-old girl's death by suicide. At on-site investigations, the young girl was found hanged from a sheet attached to the railing of her apartment. The external examination of the victim revealed the presence of hanging marks: cyanosis of the face, conjunctival petechiae, soft skin neck injury, and hypostasis in both hands and feet. The examination of the victim demonstrated suicide by hanging. A psychological survey was performed on the family and friends of the victim. Measurements used were: face-to-face structured interviews, semi-structured interviews with family members of the suicide victim, or next of kin with informed consent obtained beforehand.

Results: The results showed the victim's parents were divorced. Further psychological investigations were suggestive of sexual abuse by a parent.

Conclusions: The study emphasizes the importance of psychological autopsy to detect the reasons for suicide. This survey is important especially to identify the risk of suicide victims in relation to: suicidal setting, family dynamics, previous psychiatric disorders of the victim, and any psychiatric disorders of the family. The identification of risks enhances prevention of this phenomenon.

Suicide, Psychological Autopsy, Interview

I33 False Accusations of Sexual Abuse as a Means of Revenge in Couples Disputes

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After attending this presentation, attendees will more fully comprehend the dynamics regarding false charges of sexual abuse eveled against ex-partners as a means of revenge in Italy.

This presentation will impact the forensic science community by presenting some of the challenges that professionals in criminology and legal medicine encounter when ex-partners launch false charges of sexual abuse against the other partner. Additionally, the importance of the role that Parental Alienation Syndrome (PAS) may play in such circumstances will be presented.

False accusations of sexual abuse as a means of revenge in couple diputes when carrying out forensic-psychiatric evaluations on a minor who is the presumed victim of sexual abuse, one may come across both "false positives" and "false negatives." These may be the result of honest mistakes, or are intentionally false, and the result of manipulation and exploitation. False accusations may come about under certain conditions such as in particularly bitter cases of separation and divorce where one of the parents files charges against the other, and is well aware of the untruthfulness of them. In other cases, the adult reports sexual abuse that did not, in fact, take place, but believes in good faith that it has. Such a parent's motives are protective in nature. Investigations carried out using inappropriate techniques may result in erroneous conclusions, thus confirming abuse that had, in fact, never taken place. The goals of this study are to examine the phenomenon of false accusations of sexual abuse as a form of revenge by one ex-partner against another, and to offer recommendations as to how to avoid falling into these traps.

This research examined 75 technical consultations and expert testimonials, requested by judicial authorities, and carried out between 2003 and 2009 at the Department of Criminology of the University of Bari, Italy. These cases regarded marital unrest where intrafamilial sexual abuse of a minor was reported. Twenty-two (30%) of these reports were found to be baseless and merely a result of conflict, as well as a strategic maneuver employed by one of the partners as an act of

revenge upon the other. Of these 22 baseless charges, five involved men who accused their ex-wives and/or live-in partners. The remaining 17 cases involved charges by women against their ex-husbands and/or live-in partners. This echoes what is reported in the literature. Mothers (alienating parents) often level "virtual accusations of abuse" against fathers (alienated parents). When, on the other hand, it is the father who is the instigator or the alienating parent, the accusations are usually aimed at the new partner of the ex-wife or ex-girlfriend.

Currently, technical consultants who work with separated and conflicted families are increasingly involved in court cases that follow a characteristic pattern: one parent is accused of sexual abuse or serious maltreatment. This causes harm to the youngster and the accused parent is subsequently turned out, losing all contact with the child. It is important to bear in mind how PAS is a form of violence perpetrated on minors. A parent who alienates the other commits a form of abuse that Gardner defines as "emotional," and may result in the permanent alienation of one loving parent, as well as psychiatric disorders.[1] The estranged parent who forces his or her child into a situation of continual denigration and denial of the other parent can irreversibly damage fundamental psychological bonds. When parents become estranged, a serious deficit in parental care is always a risk and should seriously be considered by the courts when they make decisions regarding child custody. Charges of abuse represent not only an instrument of protection of one's own children, but also a weapon of revenge against an ex-partner, paradoxically putting these minors into the role of victims.

Reference:

1. Gardner, R. A. (1999). Differentiating between the parental alienation syndrome and bona fide abuse/neglect. American Journal of Family Therapy, 27(2):97-107.

False Accusations, Revenge, Sexual Abuse

I34 A Comparison of Results From Clinical and Forensic Urine Screening for Opiates in Psychiatric Patients

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After attending this presentation, attendees will learn how clinical toxicology testing is not as accurate and as specific as they should be for patients with a history of drug abuse and psychiatric illness. Attendees will also learn that many patients who are currently using opiates would return a hospital drug screen as "negative" for opiates when this clearly is not the case. The screening methodologies currently employed in clinical laboratories are not sensitive enough to provide comprehensive toxicology results.

This presentation will impact the forensic science community by improving appropriate treatments and diagnoses for psychiatric patients.

Introduction: Patients with mental illness, such as bipolar or schizophrenia, are more likely to have substance abuse problems than the general population. If both are identified, then the individual may receive continuous treatment for each affliction. One problem associated with drug abuse lies in the detection of the drugs in a clinical setting. Patients with mental illness and drug use present a difficult challenge for physicians to determine if the causation factor for the mental illness is drug abuse or if the mental illness led them to drug abuse. If drug use is suspected, a urine sample is collected and sent to the hospital laboratory for drug screening. The drugs that are routinely screened for vary between hospital and/or institution. The screening is typically five different drug classes that include: cannabis, amphetamine(s), cocaine, opiate(s), and benzodiazepine(s). In most hospital laboratories, no confirmation for drug use is performed due to the conception that this is expensive and time-consuming. The reliability and accuracy of the urine toxicology results is a vital tool for the correct diagnosis of these patients.

Objective: This study was conducted to assess the accuracy of the clinical toxicology testing for patients with a history of drug abuse and psychiatric illness. According to the data from the National Institute on Drug Abuse, the number of opiates prescribed has dramatically increased in the last 20 years, with 131 million prescriptions written/dispensed in 2000 that had increased to 210 million in 2010. Due to this increase in use and the lack of cross reactivity with some opiates in hospital drug screening, a study was conducted comparing clinical toxicology results with typical forensic toxicology screening that combines Enzyme Linked Immunosorbant Assay (ELISA) and Gas Chromatography–Mass Spectrometry (GC-MS) to screen and confirm a wide variety of drugs.

Materials and Methods: In this IRB-approved study, 338 urine samples were collected from the hospital laboratory from patients admitted into detox or mental health institutions. Patients were comprised of males and females with ages ranging between 18 and 65 years old. The results from the hospital urine drug screening with EMIT (Enzyme Multiplied Immunoassay Technique) was obtained. The forensic toxicology testing protocol utilized a DS2 (Dynex Technologies) fully automated ELISA instrument using opiate and oxycodone ELISA kits (Neogen KY, USA). The comprehensive GC-MS screening utilized a basic LLE (Liquid-Liquid Extraction) then fast GC-MS (Agilent Technologies) analysis. These tests are comparable in pricing and in speed that is vital for routine toxicology testing in a hospital environment.

Results and Conclusions: The results of both laboratories urine drug screening are presented in Table 1. With the EMIT screening technologies; they tend to have a poor cross-reactivity to oxycodone and oxymorphone which can explain some of the differences seen. Clearly, the results demonstrate that the current screening methodologies typically employed in clinical laboratories are not sensitive enough to provide comprehensive toxicology results. The results show that many patients that are currently using opiates would return a hospital drug screen as "negative" for opiates when this is evidently not the case. The screening used at the hospital at baseline for most of the patients were inaccurate and unspecific and in this instances missed over half of the patients using opiates which is important information when trying to determine the treatment for those patients. The GC-MS data identified a wide variety of opiates in use in this population use ranging from 6-monoacetylmorphine, hydrocodone, hydromorphone, oxycodone and oxymorphone to name a few.

Table 1: The drug screening results of a clinical laboratory vs. the drug screening results a toxicology laboratory. N= 338

Drug Group	Toxicology Laboratory		Clinical Laboratory	
	Number of Positive Patients	% Positive	Number of Positive Patients	% positive
Opiates (class)	30	9	22	6.5
Oxycodone (class)	20	6	N/A	N/A

Inaccurate, Opiates, Screening

I35 An Institutionalized Elderly Sexual Offender: A Case Report

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The goal of this presentation is to describe a case of sexual abuse perpetrated by an institutionalized patient toward a young visitor.

This presentation will impact the forensic science community by focusing on elderly sexual offenders in order to better understand the mechanisms and factors that lead to elder sexual offenses in both family and formal care settings.

Background: More commonly, older people are the victims of sexual abuse but they can be also the perpetrators. The phenomenon is complex, consisting of institutional mistreatment toward all residents or individual neglect toward a single patient. The reasons older adults commit sexual offenses, in particular against children, are still an unsolved problem that requires understanding the motivations and distinguishing between senile or pedophile. Such offenses can occur in the family as well as within the hospital, nursing home facility, or a residential care home for elderly. This case report describes an episode of sexual offense by an elderly institutionalized patient toward a young visitor.

Case Report: A 70-year-old man in a residential care home was accused of sexual abusing a 6-year-old girl who was with her mother to see the grandmother. In the late afternoon, the grandmother reported to staff members that the male resident had come to her room, promised the child some candy, and had then taken her with him to his room. Once back in her grandmother's room, the girl disclosed that the man had lowered her underwear and touched her private parts. The elderly man suffered from Parkinson's disease for several years, was first treated with dopamine agonists, then, for the appearance of long-term treatment syndrome and gambling, with atypical neuroleptics. During an interview, the child was able to mimic the abuse perpetrated, by using a doll, and by psychodiagnostic tests performed, she was found to be reliable in reporting the sexual abuse. On genital examination, no injuries were observed except for some hyperemia. The analysis of the micro-traces on her underwear gave negative results for the presence of seminal fluid but it was able to detect a DNA profile consistent with that of the elderly man. The man was found guilty and sentenced to five years in prison. A civil action was also brought against the staff of the nursing home for inadequate supervision of the resident.

Discussion: The elderly are often unrecognized victims of sexual abuse but also at risk of perpetrating abuse on vulnerable targets like children or frail elderly co-residents because of many factors associated with aging. Mental illness and cognitive disturbances (related to dementia, other neurological or iatrogenic factors), as well as a range of problems in addition to physiological or pathological sex behaviors (i.e., pedophilia) are just some of the several age-related factors that can lead to such offenders. Recently, evidence has been found linking antiparkinsonian therapy and disorders in the impulsive-compulsive spectrum including gambling and hypersexuality as well as dopamine dysregulation syndrome. A distinguishing characteristic of elderly sex offenders is that the illegal activity will usually take place in a private place, such as the home of either the offender or the victim, or in a hospital or residential care home. Because of the aging of our population, elderly sexual offenders are becoming an increasing concern to clinicians and criminal justice agencies.

Conclusions: It is important to have a fuller understanding of the factors leading to elder mistreatment and sexual offenses in both family and formal care settings. Caregivers have responsibilities to ensure the safety of dependent elders and to protect them from elder abuse and also to ensure they do not pose a risk to those they are living with and any visitors they may have. Moreover, it is necessary to better understand the motivations and psychological factors relating to elderly sex offenders, to prevent these offenses, and to define standards for surveillance of residents who are potential perpetrators.

Elderly Offenders, Nursing Home, Sexual Abuse

I36 Sex Offenders, Empathy, Aggression, and Oxytocin: A Potential Avenue for Future Exploration?

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After attending this presentation, attendees will gain a basic knowledge of the current state of research examining effects of oxytocin on empathy and aggression. Attendees will consider the potential avenues of future research with oxytocin and sex offenders.

This presentation will impact the forensic science community and the mental health profession by suggesting that empathy deficits and aggressive behavior in sex offenders may be partially a result of oxytocin deficits in such offenders, particularly given recent research showing oxytocin's important role in increasing empathy and modulating aggression.

Intimacy deficits, cognitive distortions, and problems empathizing with victims have been factors implicated in the genesis and maintenance of sexual offending. Sexual offenders may suffer from a deficit in the ability to identify and appreciate the emotional experiences of others. Based on this framework, the development of empathy has been a central tenant in almost all sex offender treatment programs. The general principle of this emphasis is that if offenders' empathic skills can be improved, they are less likely to re-offend. Psychotherapy is the general treatment modality used to improve empathy in such offenders, but there are several challenges to this process, depending on the offender's motivation, therapist's skill, and a host of other factors. Exploring alternative ways to increase empathy in this population is warranted. If a safe and effective treatment modality can be developed to increase empathy in sex offenders, it is possible that this form of treatment may lead to a reduced risk of reoffending.

Oxytocin is currently receiving much attention from the psychiatric community, as it has been shown to play a role in bonding, attachment, peer recognition, trust, and empathy. Dubbed the "Love Drug" or the "Hormone of Love," it is a neuropeptide that is produced in the hypothalamus and released into the brain and bloodstream. Oxytocin can be given by an intranasal administration that crosses the blood brain barrier and thus has been used in several trials on humans. Several studies have focused on oxytocin's effects on empathy, as defined by the capacity to share and understand the feelings of others. For example, oxytocin has been shown to increase emotional empathy in response to both positive and negative stimuli using the Multifaceted Empathy Test. Another study found that administration of oxytocin increased mock jurors' perception of harm for the victims but did not increase the desire to punish the offenders for their criminal offenses, thus promoting the idea that oxytocin played a role in promoting emotional empathy. There is some evidence demonstrating that oxytocin may improve the accuracy of recognizing emotional state in others, particularly in individuals who would otherwise fail to make appropriate judgments based on social cues. In addition to research examining oxytocin's effects on empathy, there is evidence to show that oxytocin is an important modulator of aggression. For example, mice in which the oxytocin gene is absent from the time of conception have shown to have heightened aggressive behavior compared to normal

mice. In humans, the levels of oxytocin in cerebrospinal fluid have shown to be inversely correlated with a life history of aggression.

This presentation will review the literature regarding research on empathy deficiencies in sexual offenders and then examine the literature on oxytocin's effect on empathy and aggression. Deficits in empathy and aggressive tendencies appear to be prominent traits in sex offenders. Given this information, combined with the growing amount of evidence indicating that oxytocin may play a significant role in increasing empathy and modulating aggression, it may be hypothesized that oxytocin deficits could be one of the contributing factors involved in the genesis or maintenance of deviant behavior in sexual offenders. Based on this review of the literature to date, future research into the effects of oxytocin on empathy and aggression in sexual offenders is warranted.

Sexual Offenders, Oxytocin, Empathy

I37 False Sexual Harassment Claims in Employment Litigation: A Framework to View Internal Incentives

Alicia L. Bales, MD, PO Box 86125, Los Angeles, CA 90086-0125*

After attending this presentation, attendees will have a better understanding that some false sexual harassment allegations may arise as a result of both internal incentives and external factors motivating the plaintiff to file a claim. Attendees will understand how psychodynamic concepts such as reaction formation, displacement, projective identification, and repetition compulsion may be factors involved in cases of sexual harassment claims that ultimately may be false in nature.

This presentation will impact the forensic science community by providing a framework for the use of psychodynamic concepts to conceptualize alternative explanations for a plaintiff's perception of events and motivations behind allegations in a sexual harassment claim.

False allegations of sexual harassment in the workplace can be filed for a variety of reasons, some of which may fall into the realm of "factitious" claims. Factitious sexual harassment claims are those in which the plaintiff's wish for victim designation is a major driving force behind the claim. Factitious disorder according to the DSM-IV-TR is the *intentional* production of physical or psychological signs or symptoms with the motivation for the behavior being primarily to assume the role of one who is suffering from a medical affliction. As a corollary, factitious sexual harassment claims are those filed with the primary goal being to assume the role of a person who has been victimized in order to experience the customary social norms that surround that role.

In addition to *intentional* factors, one could hypothesize that *unconscious* internal incentives may also be factors in the development of a false sexual harassment claim. When a forensic mental health evaluator conducts an assessment of the plaintiff, both internal as well as external incentives should be explored to help identify potential motivational factors behind the claim. Being aware of potential internal and external incentives in these evaluations may be of assistance when considering alternative explanations for the plaintiff's perception of events and alternative explanations for the allegations.

This presentation proposes the use of psychodynamic principles to aid the forensic mental health evaluator in considering conscious and unconscious components that may contribute to the development of a false sexual harassment claim. For example, individuals who have a history of prior abuse may retain a sense of victimhood that is not always validated by the external world. Filing a claim of sexual harassment places the plaintiff in the role of a victim, and inhabiting this role may validate an inner experience that had been left unresolved. This may lead to a situation where the individual unconsciously seeks out another person who is in a position to potentially act as an aggressor and with whom the individual can re-enact the abusive dynamics experienced in past relationships. In these cases, the sequence of events might be hypothesized to unfold in two parts. First, the individual finds someone to re-enact a relationship where that person conforms to the role of the "aggressor" whereby the individual sees himself or herself as the "victim" of harassment. Second, when the lawsuit is filed, the plaintiff who was originally in the role of the victim can now become the "aggressor" while the defendant is now made to experience the role of being the "victim." This situation creates a dynamic that not only fits the plaintiff's internal sense of self as a victim but then goes one step further to allow the individual a chance to "master" the present and past situations by reversing roles. This is called engaging in repetition compulsion by projective identification, which is an interpersonal process whereby the individual adapts to unwelcome feelings by evoking the same feelings in another. By filing a lawsuit, the individual may cause the perceived attacker to be the person under attack, perhaps feeling humiliated, fearful, and hurt as that which the individual once experienced. The concepts of reaction formation, projective identification, displacement, misattribution, and reaction formation will be used as a framework to explore potential internal incentives operating in a plaintiff to bring about such claims.

Sexual Harassment, Factitious, Psychodynamic

I38 Cyber-Sexual Harassment and Adolescents

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The goals of this presentation are to: (1) understand definitions of cyber-sexual harassment; (2) learn the prevalence of adolescent Internet usage and online media as well as prevalence and effects of cyber-sexual harassment on adolescents; and, (3) become familiar with methods of risk assessment and intervention strategies to reduce cyber-sexual harassment among adolescents.

This presentation will impact the forensic science community by outlining the scope and effects of adolescent cyber-sexual harassment and suggest methods of risk assessment and intervention strategies for this important and widespread problem.

Recent attention to cases of suicide among youth victims of cyber-sexual harassment has generated interest in its prevalence, correlates, and psychological impact. While face-to-face sexual harassment is a well-known problem, understanding of the scope and implications of online or cyber-sexual harassment is emerging. Cyber-sexual harassment also is a widespread problem among youth. Adolescents have wide access to the Internet. Pew Research Center reports indicate that 93% of teens have access to the Internet and 75% have their own cell phone.[1] According to a recent survey of internet-using 10 to 15-year-olds, 33% reported online sexual harassment in the prior year, 15% reported an unwanted sexual solicitation online in the same time period, and 4% reported a sexual solicitation incident on a social networking site specifically.[2]

Cyber-bullying, generally, and cyber-sexual harassment, specifically, affect youth negatively in multiple domains. Cyber-bullying has been associated with poor school performance and psychological distress. Recent studies of cyber-harassment among middle and high school students have found that cyber-bullying is associated with poor grades in school, lower odds of planning to attend a four-year college, depressive symptoms, suicidal ideation, and self-injury as well as suicide attempts.[1,3] Being a victim of cyber-harassment was also associated with hardcore and softcore drug use and drunk driving, skipping school, and school suspension, as well as gang membership and carrying a weapon.[3] Non-heterosexually identified youth appear to be victims of cyber-bullying more frequently than heterosexually identified youth, girls more frequently than boys.[1] In offline harassment among adolescents, victimhood can predict later perpetration, or they may co-occur.[4] It is not known whether the same pattern exists in cyber-harassment or cyber-sexual harassment.

This presentation will focus on cyber-sexual harassment with respect to adolescent offenders and victims. Definitions of cyber-sexual harassment, prevalence data, and comparison with offline bullying will be discussed. Similarities and differences between offline

bullying/sexual harassment and its cyber counterpart will be considered, including whether the latter may be considered a category of sexual offending. An overview of offline sexual harassment, including landmark legal cases, will be given.

Prevalence of adolescent internet usage and online media most relevant to cyber-sexual harassment (e.g., social network sites like Twitter, Facebook, postings on YouTube, communication via email/texting) will be covered. There will also be a discussion of developmental aspects of this behavior, methodologies for assessment of risk, and intervention strategies to reduce cyber-sexual harassment among adolescents. Case studies will be used to illustrate examples.

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Adolescents, Sexual Harassment, Cyber-Sexual Harassment

I39 Characteristics of Child Pornographers: A Description of a Cohort Subjected to Forensic Evaluation

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After attending this presentation, attendees will obtain information about child pornographers, online sex offenders, and hands-off offenders. The goal of this study is thus to identify the characteristics of people who are drawn to child pornography.

This presentation will impact the forensic science community by displaying the characteristics of an offense which increased because of the Internet. The characteristics of the classical pedophile is not what we find in child pornography.

Introduction: Child pornography is a form of sexual exploitation of children. It depicts erotic or sexual scenarios with children, either explicitly or implicitly, nurturing individuals' deviant fantasies or facilitating a move toward pedophilia. Seeking and collecting images is never a matter of chance, and the collecting process and the way the pornographer considers his collection need to be better understood. It is these psychopathological issues and the risk of the person acting out his fantasies that need to be analyzed. The use of telecommunication facilities, particularly the internet, has allowed child pornography to be distributed more widely.

Today, access to pedophilic material no longer requires direct contact between supplier and consumer. The phenomenon has increased through the use of peer-to-peer networks, which make it easier to receive images. Exchanges between collectors permit them to become part of groups that gives them a virtual social life. Some believe that downloading images does not make them criminals because they were not present at the time of the abuse. Understanding the phenomenon of child pornography is complicated, because it is sometimes a matter of deviance without leading to actual abuse, and it is this virtual aspect that appears to be encouraged by Internet. It has probably increased certain deviant behaviors that were previously limited due to inhibition.

The main question facing law enforcement agencies is the level of danger posed by child pornographers. In essence, judges need to know the likelihood that sexual abuse will occur. A number of elements indicating a risk of child sexual abuse have been identified, including participation in a virtual community, sharing images, and possession of new images, but more elements are needed for a better understanding of this problem.

Material and Method: This study was based on pre-sentencing forensic psychiatric assessments carried out at the request of the courts. The forensic psychiatric assessments have been conducted by different forensic psychiatrists in France. The main criterion for inclusion was the possession, distribution, and/or production of pornographic images of minors, and that the subjects acknowledged the charges.

Various psycho-socio-demographic elements were studied based on evidence gathered from forensic psychiatric assessments and data from the literature (e.g., sex, academic level, marital status, job, social integration, childhood, type of offense, attitude toward the offense, criminal record, addiction to images, pedophile tendency, sexuality, introspection, medical history, dominant personality traits, diagnosis, therapy).

Discussion: Child pornography is a complex phenomenon. It generally fulfills a fantasy of a pedophile nature. Different profiles of child pornographers have been proposed which help understand underlying deviant motivations, which are not always related to pedophilia. Internet offenders can be divided into those who seek online contact and those who purchase offline contacts. While the socio-demographic characteristics of these subjects have been identified, further psycho-socio-criminological elements are needed to determine the profiles in greater depth.

The sample matched the descriptions found in the literature. Subjects were almost exclusively male with an average age of forty years, which is consistent with studies that have generally reported an age range of 25 to 50 years. They appear to be younger than offline offenders. In this study, the inclusion of a 15-year-old indicates that child pornography is not just about age.

Over 80% had a diploma, and over 50% had studied at university. Half of the cases had a stable job. Overall, the data suggest that all the subjects had a sufficient level of education to pursue a career and fit into society. However, a third of the cases felt that they were not well integrated, although half of those had a stable job. Moreover, they did not consider that living with a partner was a factor of good social integration.

There is no data in the literature about the sexuality of child pornographers. Most individuals in the study were heterosexual men, and most expressed poor sexual satisfaction, although more than half lived with a partner. Most of the homosexuals in the study were single and expressed more sexual dissatisfaction than the heterosexual subjects. Living with a partner does not necessarily lead to sexual satisfaction.

Forensic Psychiatry, Child Pornography, Sex Offenders

I40 Replicating the Seigfried-Spellar and Rogers (2011) Study on Deviant Pornography Use by Age of Onset and Sex

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After attending this presentation, attendees will have a better understanding of the relationship age of onset, sex, and pornography use. In other words, individuals who engage in adult pornography use at a younger age are at a greater risk for engaging in deviant pornography use (e.g., child porn), and whether sex is related to deviant pornography use. In addition, attendees will have a greater appreciation for the importance of empirical replication.

This presentation will impact the forensic science community by providing more information regarding the relationship between non-deviant and deviant pornography use. This study is a replication of a previous one which will extend and validate the previous conclusions of the Seigfried-Spellar and Rogers study.[1]

Although seemingly counterintuitive, research indicates the collections of child pornography users not only contain sexualized

images of children, but other genres of pornography both deviant and socially acceptable in nature.[2-3] In fact, interviews with child pornography users have suggested that some offenders move "thorough a variety of pornographies, each time accessing more extreme material" as a result of desensitization or appetite satiation, which lead to collecting and discovering other forms of deviant pornography.[2-3] Also, some consumers stated they downloaded the images simply because they were available and accessible, making the behaviors primarily a result of compulsivity rather than a specific sexual interest in children.[4]

Some child pornography consumers exhibit a complex array of sexual interests, which may be representative of a more general level of paraphilic tendencies rather than a specific sexual interest in children. In a study conducted by Endrass et al., the collection of images from 231 men charged with child pornography use also revealed other types of deviant pornography.[5] Specifically, nearly 60% of the sample collected child pornography and at least one other type of deviant pornography, such as bestiality, excrement, or sadism, with at least one out of three offenders collecting three or more types of deviant pornography.[5] This research suggests the majority of Internet child pornography users are collecting a wider range of deviant pornography, which may reflect a general level of sexual deviance rather than a specific paraphilia, such as pedophilia. In other words, some child pornography consumers may be dissidents within the normal population who exhibit a wider range of sexual interests.

The Seigfried-Spellar and Rogers sampled a panel of United States Internet respondents from the Survey Sampling International, Inc.[1] The respondents were asked their sex, age of onset for various forms of non-deviant (e.g., adult pornography) and deviant pornography (e.g., child pornography), and their level of engagement in Internet pornography (e.g., searching, downloading). Six hundred and thirty respondents completed the online survey; 33 (5%) of the sample self-reported engaging in child pornography. Results indicated that people who used adult pornography were significantly more likely to use animal and child pornography. In addition, age of onset for non-deviant pornography use (adult-only) was significantly related to deviant pornography use (child, animal). In other words, individuals who engaged in adult and deviant pornography had a significantly lower "age of onset" compared to individuals who only engaged in adult pornography.

However, this study was the first to assess whether age of onset and sex were related to deviant pornography use; thus future replications are needed to determine the validity of the findings. First, the current study will utilize a snowball sampling method by soliciting Internet respondents from various Internet websites rather than a paid panel sample of U.S. Internet users. In addition, the respondents will not only be permanent residents of the U.S. but the sample will include respondents from Canada, Australia, and United Kingdom. Again, this study will assess whether *age of onset* is a risk factor for engaging in deviant pornography. In other words, are individuals who engage in nondeviant pornography use at an earlier age more likely to engage in deviant forms of pornography use compared to late onset users? In addition, this study will explore the frequency of pornography use by collapsing the respondents into pornography categories: none, adult-only, animal-only, child-only, adult-animal, adult-child, animal-child, and adult-child-animal. This aim will assess whether self-reported child pornography users are more likely to self-report adult and animal pornography behaviors compared to the other categories of users.

Finally, a comparison in results will be made to the original Seigfried-Spellar and Rogers study to determine the validity of inferences drawn from the original findings.[1] A detailed discussion of the study's results and future implications will be discussed.

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Deviant Pornography, Age, Sex

I41 Use of Clinical Mental Health Professionals in Transition Adjustment Programs for OEF/OIF Veterans as New Police Officer Recruits

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The goals of this presentation are to: (1) provide a cognitive structure that explains how this kind of project is expected to contribute to the reframing and reorganization of police officer's ability to receive information relevant to stressful transition associated with the new civilian department; (2) communicate findings that will suggest important implications for mental health transition services specifically-related to Operation Enduring Freedom/Operation Iraqi Freedom (OEF/OIF) veterans; and, (3) create a foundation for additional research on this topic.

This presentation will impact the forensic science community by describing the clinical mental health student component of a military-police cultural transition model being implemented in a large west coast police department.

There are potential negligent hire and retention risks associated with OEF/OIF veterans as new police officers. For some police departments, there has been tremendous growth (i.e., anywhere from 30 to 50 percent) in the proportion of these veterans moving into civilian police ranks. While their presence is welcomed, it has also focused increased attention on concerns about post-deployment Mild Traumatic Brain Injury (mTBI) and Posttraumatic Stress Disorder (PTSD). For example, PTSD has a predictable unwanted effect on the life course of veterans that is marked by both physical and psychological issues. A problem made worse by the presence of other psychological conditions that include the combined effects of dysphoria, as well as mTBI. Alcohol and other forms of substance abuse are also a part of the clinical picture of these veterans who are probably at a higher risk for premature departure from the military and quite vulnerable as new police recruits.

The OEF/OIF veteran clinical problems mentioned above are oftentimes inextricably linked to other sources of psychosocial stress. For example, financial pressures can occur from having psychological disorders like PTSD or other mood disorders. Most notably, they can create money-management problems, a situation made even worse if this OEF/OIF veteran had pre-existing problems managing their financial affairs. Pre-employment physicals and psychological screenings may not capture the full scope of the issues presented by these veterans. Fueled by budget cutbacks due to a declining economy, police departments are forging university partnerships with faculty that have experience in police psychology. Successful transition from the military to civilian life is important. Equally important are the transitions required from the military to police culture. The transitions from military to civilian life are exacerbated by the stress of trying to secure a job, family stress, and other financial pressures from a depressed economy. A new career as a police officer sounds like a relief, but may in many respects represent another stress on an OEF/OIF veteran who has already

carried the burden of post-deployment stress. Moving from the stress from military service, to the stress of post-military life, and then into the stress from the police world which may further compromise an already distressed OEF/OIF veteran. Some departments are fully aware of these OEF/OIF issues and have implemented resources intentionally aimed at the issues that they present as new police recruits.

The first learning objective of this presentation is to explain a cognitively-oriented approach for this OEF/OIF veteran group. This approach explains how this kind of OEF/OIF veteran-police recruit project is expected to contribute to the reframing and reorganization of police officer's ability to receive information relevant to stressful transitions associated with the new civilian department. The second learning objective is to communicate findings that will suggest important implications for mental health transition services specifically-related to OEF/OIF veterans. The third objective is to create a foundation for additional research on this topic. This presentation describes the clinical mental health student component of a military-police cultural transition model being implemented in a large west coast police department. The community benefits by decisions made to create resources that support OEF/OIF involved in civilian public safety.

OEF/OIF Veterans, Military Transition, Police Officer Recruits

I42 Collaborations Between Clinical Mental Health and Police Wellness Units: A San Diego Police Department Project

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After attending this presentation, attendees will be made aware of the need to extend the support network by forging relationships with university-based mental health professionals with expertise in police psychology. At the same time, wellness unit staff must remain sensitive to the process of orienting new police officers. For example, what is not as well-known is that new police officers are probably more receptive to using department support resources early in their career as opposed to after they have been absorbed into the police culture. Attendees will also be briefed on the Peace Officer Standards of Training's (POST) psychological dimensions as an evidence-based anchoring base for mental health professionals to use while making the risk-assessment rating. A practice-relevant forensic case study is used to demonstrate how the POST psychological dimensions can be used in this risk-assessment process. It is hypothesized that attendees from this presentation will have a greater understanding of the forensic elements associated with use of the psychological dimensions of police officer functioning.

This presentation will impact the forensic science community by providing knowledge of the enhanced performance of police officers who make use of the combined resources offered through the wellness unit.

Collaborations between qualified university-based clinical mental health professionals and police wellness units offer a reasonable approach to dealing with risks (e.g., death, PTSD, suicidal behavior) police officer psychological work. Police officers have the most stressful job of any occupation. Risk assessments in the context of police work refers to the identification and psychological weighing of potential factors that are expected to compromise judgment or increase concern about stress vulnerability that can fuel misconduct. Despite the obvious need for services and available department resources, wellness units must reflexively work to achieve delicate cultural balance. The balancing act is required for negotiating the meandering contours of a police culture. The police culture includes an instinctive tendency for a male-dominated culture to avoid any revelation of what they assess as a weakness, especially those highlighted by mental health. Second, police unions are extremely leery (i.e., anonymity, fears of department, retribution, or any potential police officer negative outcomes) of any department resource (e.g., EAPs, chaplains, or senior officer mentors) that brings their members under scrutiny where they might be perceived vulnerable for being singled out. Third, the wellness unit is saddled with an onerous task of finding creative ways to assist police officers who they know are struggling with a wide range of issues. Finally, department budget cutbacks have resulted in loss of critical professional staff with the requisite expertise in responding to the stress-based concerns present by police officers.

The complexity of these police wellness issues underscores the diversity of public safety challenges. Most of these challenges on the surface appear rather obvious as to what may be required to intervene. Yet, with considerably more understanding of the dynamics of police culture, it becomes clear that gaining access to assist must be done with a unique or tailored model for a specific police department. For example, law enforcement agencies have distinct cultures (e.g., U.S. Border Patrol versus Boston Police Department) that must be first properly assessed in order to gain access for intervention. The hallmark of this work must first demonstrate the feasibility of working with the male-dominated culture posed by an otherwise hard-to-reach police population. This process is expected to be aided by understanding the beliefs and behaviors necessary for developing appropriate risk prevention and intervention programs in a department. University clinical mental health faculty with experience in working with police and public safety can be quite productive in crafting such collaborations assuming 100% buy-in (i.e., top-down cultural change) by the chief or superintendent.

In the aftermath of a high profile police misconduct incident, once the incident details are disclosed, it becomes clear that there are matters that should have previously raised red flags for the department. Police suicides are also devastating to the morale of other officers. The financial costs to departments and municipalities for a police officer's improper action reinforce the need to reduce the law suit risks where negligent hire can erode public trust. Insurance rates are also expected to rise in the wake of such cases. There are three learning objectives associated with this presentation. First, attendees are made aware of the need to extend the support network by forging relationships with university-based mental health professionals with expertise in police psychology. At the same time, wellness unit staff must remain sensitive to the process of orienting new police officers. For example, what is not as well-known is that new police officers are probably more receptive to using department support resources earlier in their career as opposed to after they have been absorbed into the police culture. The second learning objective of the presentation uses the POST's psychological dimensions as an evidence-based anchoring base for mental health professionals to use while making the risk assessment rating. The author uses a practice-relevant forensic case study to demonstrate how the POST psychological dimensions can be used in this risk assessment process. This presentation articulates a model of collaboration between university-based clinical mental health and police departments. The authors hypothesize that attendees from this presentation will have a greater understanding of the forensic elements associated with the use of the psychological dimensions of police officer functioning. The community is expected to gain from the enhanced performance of police officers who make use of the combined resources offered through the wellness unit.

Collaborations, Mental Health, San Diego Police

J1 Determination of the Age of Ink Entries From Questioned Documents With TD-GC/MS and HPLC Methods

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After attending this presentation, attendees will learn how an ink entry on a questioned document is related with entrry date.

This presentation will impact the forensic science community by demonstrating how ink age determination can be accomplished using the dynamic physicochemical properties of the ink entries on a document.

Determination of the age of an ink entry from a questioned document is a difficult and controversial issue in forensic science. Lately, the studies of ink age determination done from the dynamic properties of the ink entries have shown that the phenoxy ethanol, which is one of the solvents in ink, has different behavior against the varying thermal conditions by Thermal Desorption and Gas Chromatography/Mass Spectrometry (TD-GC/MS) during methyl losses of the pigments and presents a valuable confirmatory evidence of age using High-Performance Liquid Chromatography (HPLC).[1]

The goal of this study is the enhancement of the ink age determination methods using dynamic physicochemical properties of the ink entries on a document such as the vanishing rate of phenoxy ethanol (PE) with TD-GC/MS that is used in traditional analyses of volatile organic components and the fading rate of the pigments Crystal Violet (CV), Methyl Violet (MV), Tetramethyl Para Rosaniline (TPR), and other changes in pigment constitution.

For comparison of thermal desorption properties, the sample cut in 0.5cm in length, was placed in a completely empty and clean thermal desorber tube. The sample was analyzed in two different runs for different temperatures: 90°C and 200°C.[2] The sample was desorbed for 20 minutes at 90°C. Then the sample was trapped at -10°C. The temperature of trap was increased to 300°C. The sample was held approximately three minutes then it was given into GC. Temperature of the transfer line was 140°C. The DB-VRX column used was 60m long and had an internal diameter of 0.25mm and film thickness of 1.4 µm. The oven temperature was programmed as follows; 45°C for one min, then from 45°C to 100°C at a rate of 30°C/min and from 100°C to 190°C at a rate of 12°C/min. It was held at 190°C for four minutes then increased to 200°C at a rate of 50°C/min and held for five minutes. To ensure better quantitative accuracy, the Selected Ion Monitoring (SIM) mode was employed, due to its higher sensitivity. The chosen quantifier ion m/z is 138,10 and qualifier ion 94,0. The same sample was desorbed for five minutes at 200°C. The system conditions chosen for 200°C was the same as the one chosen for 90°C. The age of ink entry was calculated by the help of the relation between the integration values at 90°C and 200°C (M_{90}, M_{200}).

For the comparison of the time dependent methyl losses of the ink pigments on the same documents, two 1.2mm punches were extracted by methanol for 15 minutes and than 10 micro liters of extract were injected to the HPLC. The chromatograms of the questioned inks were compared to find age differences in between.

By the present methods proposed, as a result of one year work and approximately 300–350 experiments the aging curve of ink (V%) was plotted and the feasibility of ink age determination in the laboratory clear. The HPLC chromatograms have been used for prediction and/or confirmation. Two examples of the use of the proposed method in caseworks is given.[2,3]

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Ink Age, HPLC, TD-GC/MS

J2 The Use of Attenuated Total Reflectance Fourier Transform Infrared Spectrometry (ATR-FTIR) in the Analysis of Paper

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After attending this presentation, attendees will understand the potential for the use of the Attenuated Total Reflectance Fourier Transform Infrared Spectrometry (ATR-FTIR) spectrometer in the field of forensic science in discriminating different brands of white multi-use and copier paper. Attendees will also understand the use of principal component analysis (PCA), hierarchal cluster analysis (HCA), and discriminant analysis (DA) in analyzing infrared spectra.

This presentation will impact the forensic science community by introducing questioned document examiners and other forensic scientists to the power of ART-FTIR combined with multivariate statistical methods such as principal component analysis and linear discriminant analysis.

Determining the sources of sheets of paper can facilitate in cases involving fraudulent documents, threatening letters, ransom notes, and other cases of questioned documents. A number of methods have been applied to the analysis and comparison of paper samples, including transmitted light imaging, laser speckle imaging, light microscopy, scanning electron microscopy, X-ray diffraction, pyrolysis-gas chromatography, Raman spectroscopy, ultraviolet-visible-near infrared spectrometry, FTIR, and ATR-FTIR. Some of these methods require the destruction or significant alteration of the sample examined. Some of the methods are also lengthy and time-consuming. ATR-FTIR has many attributes that commend its use for the analysis of paper: it is non-destructive; it can analyze small areas (thus avoiding areas of paper which bear printing or writing); it requires virtually no sample preparation; and, it is very fast (individual spectra being obtained in under one minute). Moreover, ATR-FTIR instruments are becoming more widely available in forensic science laboratories.

ATR-FTIR has been applied in the past to paper pulps and to a limited number of finished papers. This study involved the analysis of ten reams of white multi-use and copier paper, representing seven different brands. The brands of paper were ones that are widely available in office supply stores, big-box department stores, and online. These brands are those that would be encountered in many offices and homes, in use as computer printer paper, copy paper, or stationery. Such papers consist of cellulose, often with fillers such as calcium carbonate, sizing agents such as starch, and optical brighteners (fluorescent compounds that contribute to the whiteness of sheets of

paper). All of these components have infrared absorptions in the range 600cm^{-1} to 4000cm^{-1}. Samples taken from each ream included the top five, middle five, and bottom five sheets. Each paper sample was pressed against the ATR crystal of the FTIR spectrometer and a spectrum was scanned. The spectra obtained from this procedure were then converted to/from percent transmittance units and absorbance units. Only the infrared region from 600cm^{-1} to 1800cm^{-1} showed significant absorption peaks; therefore, only this region was analyzed further. Some of the infrared spectra displayed anomalous baseline offsets. To deal with these, all of the spectra in the 600cm^{-1} to 1800cm^{-1} range were smoothed and then converted to first derivative spectra using the Savitsky-Golay method. The first derivative spectra from 604cm^{-1} to 1300cm^{-1} were analyzed using principal component analysis (PCA), hierarchal cluster analysis (HCA), and discriminant analysis (DA). Features of interest in the first derivative spectra were confined to this spectral range.

It was observed that there the infrared spectra displayed a high degree of consistency within a ream of paper. In PCA eight factors were required to account for more than 90% of the variance in the data. The factor plot of the first two extracted factors indicated that the ten reams of paper could be placed in four groups: a group of seven and three groups of one each. DA using the first eight extracted factors and these four groups resulted in 94.3% correct classification of the infrared spectra and 93.8% correct classification on cross-validation. Two paper brands could be differentiated from the others and two different grades of paper belonging to the same brand could be differentiated from each other. While ATR-FTIR proved not be highly discriminating among paper brands, the simplicity of the technique, its non-destructive nature, and the increasing availability of ATR-FTIR instruments make this technique a useful addition to the methods of paper analysis.

Paper, FTIR, Chemometrics

J3 The Forensic Language-Independent Analysis System for Handwriting Identification (FLASH ID)

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After attending this presentation, attendees will have an understanding of the purpose and capabilities of FLASH ID.

This presentation will impact the forensic science community by providing information on a new system that can facilitate research on handwriting examinations and expedite handwriting comparisons involving voluminous evidence.

The Forensic Language-Independent Analysis System for Handwriting Identification (FLASH ID) is an automated handwriting comparison system that allows document examiners to compare voluminous pages of handwriting in a fraction of the time it would take using conventional manual methods. FLASH ID uses graph theory to measure topographic and geometric characteristics in handwriting and generates a confidence score that is used to rank suspect writers (similar to the Automated Fingerprint Identification System). This ranking is then utilized by document examiners to narrow down the list of potential authors of the questioned writing, thereby potentially eliminating the need to compare the writing of every writer in a given database. This software could prove useful for cases similar to the Weinberger kidnapping of 1956, whereby it took a team of people over six weeks to examine nearly two million pages of handwritten public records before identifying Angelo John LaMarca as the writer of the ransom notes. FLASH ID "sees" writing as graphemes, not characters, and therefore can conduct comparisons on any language. Operating FLASH ID begins by either scanning a document in or selecting one from a file for comparison against a pre-loaded database. Once the metadata for the selected document has been entered, the user selects one or multiple databases for the document to be compared. Depending on the size of the database(s) and the performance of the computer, this could take anywhere from 30 seconds to hours. After the comparisons are complete, the ranked results are displayed along the right margin, and the user can double click on any of the documents for side-by-side view. Other select features of the FLASH ID user interface include the ability for users to select regions of interest for comparison, load multiple documents at one time for comparison (bulk load), and view the "heat map" of a document, which color-codes the strongest areas of similarity between the questioned and known writing. Current challenges include successfully extracting "clean" handwriting from a document for comparison. This is currently accomplished using over-the-counter image processing software; however, FLASH ID provides a "line removal" tool that eliminates concentric lines typically found on ruled notebook paper. Preprocessing of document ensures that scores are based on the comparison of handwriting and not extraneous markings or background noise on the page. Another challenge is interpreting meaning from the confidence scores. If a document scores relatively higher than the next closest score, then this can be interpreted that there is a significant amount of distinction. However, because each comparison depends on the quantity and style of handwriting, the scores cannot yet be used to establish a unilateral threshold for identity versus non-identity of handwriting. For the field of document examination, FLASH ID is a significant step toward the quantification of handwriting comparisons, and can be an invaluable tool for further research and validation of the principles of handwriting identification. For the forensic document examiner, FLASH ID has the potential to facilitate expeditious examinations of voluminous evidence, and may some day be used for objective verification of conclusions.

FLASH ID, Handwriting, Handwriting Analysis

J4 Stone Paper: An Overview of its Characteristics and the Impact They May Have on Forensic Document Examinations

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After attending this presentation, attendees will become familiar with a few of the stone papers (FiberStone®, Gartner®, Oxford®, TerraSkin®, ViaStone™) available on the commercial and retail markets for purchase. Attendees will understand the limitations involved in stone paper exams as well as what modifications may be needed when conducting certain questioned document examinations. These examinations include: indented impressions, typewriting, non-destructive ink (writing instruments, toner and inkjet printing) examination, tearing, folding, and stapling. The information gleaned from the latter three studies may be useful in alteration cases. An overview of water and grease interaction with inks on the stone paper, as well as the effect of environmental exposure on stone paper, is also discussed.

This presentation will impact the forensic science community by increasing the depth of knowledge regarding stone paper, a relatively new product available for purchase that may eventually appear in casework. Stone paper's general characteristics and modifications or work-arounds that are needed during questioned document examinations will be discussed. The goal is that this presentation will encourage members of other disciplines to develop their own studies regarding whether or not special considerations need to be taken for cases involving stone paper.

Stone paper (FiberStone®, Gartner®, Oxford®, TerraSkin®, ViaStone™) is comprised mainly of calcium carbonate and plasticizers. Unlike pulp paper, it is manufactured without large amounts of water and chemicals. Due to its unique composition and manufacturing process, stone paper manufacturers claim it is: more environmentally friendly than traditional pulp paper, 100% recyclable, water, grease, and tear resistant, and bio-degradable. Stone paper has been commercially available for ten years and is used by the packaging and marketing industries as gift bags, boxes, labels, and other wrapping materials. Many of these stone paper products are touted as being "greener" and, therefore, are used by a few environmentally conscious companies

whose products can be found on store shelves. In the past three years, stone paper has become increasingly available in household consumer-use products, including notebooks and inkjet photo paper, which are available at various office supply, novelty, and "big box" stores, among other sources. For this study, supplies were obtained through manufacturer donations and purchase. As stone paper becomes more available, the forensic community will likely encounter stone paper in casework.

This study seeks to introduce and educate the forensic document community and the forensic science community at large, about stone paper characteristics, evaluate some of the manufacturers claims outlined above, and describe special considerations that must be taken during forensic document examinations involving stone paper. Actions and associated examinations undertaken include: indented impressions, typewriting, non-destructive ink (writing instruments, toner and inkjet printing) examination, tearing, folding and stapling, water and grease interaction, and environmental exposure. Results of the current study will show that examination of stone paper can be conducted in a manner similar to traditional pulp paper, with a few exceptions regarding indented impression examinations and fracture matching.

Stone Paper, Paper Examination, ViaStone™

J5 The Case of Different Documents and Different Conclusions

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The goal of this presentation is to show why the reports of two different Forensic Document Examiners (FDEs) cannot be compared if they did not examine the same exact writings.

This presentation will impact the forensic science community by demonstrating how the examination and comparison of different writings on purportedly the same documents can lead to different conclusions. This is especially true when one set of the questioned documents was fabricated using the second set of documents, eventually examined by another laboratory, as the models for the creation of the first self-serving set of documents.

This case originated when Dennis "Chip" Harrup's company, Central Virginia Aviation, sold an aircraft and led the buyer to believe that the annual airworthiness inspection had been completed. Upon delivery, the aircraft did not contain the FAA mandated aircraft maintenance records. Mr. Harrup assured the buyer that the airworthiness inspection had been done. The FAA requested Mr. Harrup send them the maintenance paperwork on the airplane. Mr. Harrup sent five forms to an FAA inspector in Memphis and the inspector made copies of those forms (unknown to Mr. Harrup) before returning them.

Later, the FAA again requested the forms from Mr. Harrup, who sent the FAA photocopies of the documents, claiming that the originals had been lost. The second set of forms sent to the FAA contained signatures different from those on the original forms. The signatures on both sets of documents sent to the FAA were in the name of Tracey Helvey, the mechanic who purportedly performed the work on the airplane.

The FAA submitted the first set of photocopies, made by the Memphis FAA Inspector, along with undictated (collected) signatures of Mr. Harrup and Mr. Helvey, to the FBI Laboratory for a handwriting comparison. After conducting the handwriting examinations, he issued an opinion that no conclusion could be reached regarding Mr. Harrup preparing the questioned signatures due to the very limited amount of comparable known writing submitted for examination. The FDE also issued an opinion that there were limited characteristics to indicate Mr. Helvey may not have prepared the questioned signatures imaged on the submitted questioned documents.

Upon receiving the forensic document examiners report, the FAA contacted the FBI Laboratory and requested assistance in obtaining exemplars from Mr. Harrup. The FDE traveled to Richmond, Virginia and briefed the FAA personnel on what type of exemplars to obtain, how to obtain them, and to not let Mr. Harrup see the questioned writing before or during the collection of the exemplars. The FAA collected over forty exemplars from Mr. Harrup. They also collected over 40 exemplars from Mr. Helvey on a separate occasion.

The exemplars from Mr. Harrup and Mr. Helvey were then submitted to the FBI Laboratory for another handwriting comparison to the first set of photocopies of the questioned documents. After conducting this examination, the FDE issued an opinion that there were significant characteristics in common between the image of the questioned signatures and the known writing of Mr. Harrup, indicating that Mr. Harrup may have prepared the questioned writing. Additionally, characteristics were observed to indicate that Mr. Helvey may not have prepared the signatures imaged on the questioned documents.

The attorney for Mr. Harrup contacted a second FDE about examining some documents in the case. The documents submitted initially were photocopies of marginal quality. The questioned signatures were nothing more than a sawtooth style of writing with virtually no significant characteristics, qualities, and features. Additionally, each submitted questioned document consisted of the signature portion of two different documents. The known writings consisted of both copies and originals by two different writers.

The examination and comparison of the writings was significantly influenced by the quality of the copies of the questioned writing. Portions of the pre-drawn baseline and questioned signatures appeared to be missing. Additionally, the spelling of the name of the signer printed below the pre-drawn baseline was different than the spelling of the name of the submitted known writer by that name. The reason for their absence could not be accurately determined with the available copies. One of the factors considered was alteration of the writing; another was the generation of the copies.

The result of the examination and comparison of the questioned and known signatures was inconclusive. No evidence of significance was noted to indicate that either of the known writers wrote the questioned signatures.

Later, a second submission of five questioned documents was received for comparison to these new questioned signatures. The examination and comparison of the questioned signature on these resulted in the conclusion that Mr. Harrup probably wrote the questioned "Tracy Helvey" signature on those documents. A verbal conclusion was reported to the attorney and he did not want a written report.

This case describes the pitfalls associated with the examination of copies of unknown generation, evidence on some copies that could be associated with more than one possible explanation for its occurrence, and some of the reasons for two forensic document examiners reaching what some could say are different conclusions. In every case where documents are examined and a report is written, only reports based on exactly the same evidence can be compared with each other. If both forensic document examiners are reviewing some of the same documents together with different documents, it cannot be expected that the conclusion reached will be identical. If both forensic document examiners are examining exactly the same documents, they should come to virtually the same conclusion, based on the evidence within the writings examined.

Handwriting, Documents, Conclusions

J6 The Forensic Examination of Non-Original Documents and Images: Is It Reliable to Make Conclusions About the Printing Process and the Type of Ink Used to Create the Original Document?

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The goals of this presentation are to: (1) determine if a Forensic Document Examiner (FDE) can reliably make conclusions about the printing process and the type of ink used to create the original document when presented with copies and image files; and, (2) determine what impact file format and resolution have on text-based documents.

This research will impact the forensic science community by providing more information about non-handwriting features that can be assessed from a non-orginal document.

Sometimes, a FDE is provided with copies and/or digital images of an original document that may not be available for examination. There have been extensive discussions in the literature regarding possible limitations when conducting examinations to determine authorship, but very little focus on non-handwriting features such as printing processes and ink type. Specifically, copies, low quality, or monochrome images, and Portable Document Format (PDF) documents are often discounted as insufficient if information is requested about the creation of the original document. Images of text-based documents may be submitted for examination and can be captured in various formats (e.g., PDF, GIFF, TIF, JPG, BMP) at different resolutions. The objective of this study is to determine if an FDE can reliably make conclusions about the printing process and the type of ink used to create the original document. Moreover, what impact does file format and resolution have on text-based documents.

A series of documents were created using various types of laser printers, photocopiers, and inkjet printers bearing ballpoint inks, roller ball pens, gel pens, and felt tip writing instruments. From the original images, copies and scans were produced using various file formats and resolutions. Physical characteristics of the copies and images were evaluated visually and using stereomicroscopy to determine if there were any gross morphological differences in the printing processes and writing inks. Finally, the documents created using the various file formats were compared to ascertain features that should be considered when rendering a conclusion.

The production of digital images and PDF documents in the course of forensic document examinations is becoming more common due to electronic storage of documents. Indeed, there is information that can be obtained from a computer forensic examination, but information about printing processes and writing inks used for the original document is in the prevue of an FDE. Depending on the circumstances in a case, the evidence that can be gleaned from this type of examination can be extremely probative.

Non-Orignal Document, Ink, Copies

J7 Role of Automation in the Forensic Examination of Handwritten Items

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After attending this presentation, attendees will be updated on the research on automation of examination procedures.

This presentation will impact the forensic science community by providing the status of research regarding automation of a forensic handwriting analysis.

The most common task in Forensic Document Examination (FDE) is the examination of handwritten items. Procedures for handwriting examination developed by the FDE community have been refined over several decades, and described among several standards published by the American Society for Testing and Materials (ASTM). During the last few years, the artificial intelligence community has also developed several software automation tools for FDE. Some of these tools attempt to replicate human FDE procedures, e.g., for determining whether a given handwriting specimen can be attributed to the writer of the known writing. As with expert systems in other manually intensive procedures, such as medical diagnosis, current automation tools are useful only as part of the overall procedure. Incorporating the computational approach within the standard FDE procedure not only places these tools in context but also helps validate and improve existing manual procedures. The standard work flow in FDE of handwritten items is considered and the steps where automation is available or possible is annotated. In this instance, the well-known Lindbergh kidnapping case is considered. The case involved multiple, handwritten ransom notes which were tested for the presence of multiple writers on the same, and all, documents; determining whether the writing is disguised; consideration of the effect of comparing formally-written known writing with informally-written questioned writing, etc.

The necessary groundwork has already been laid down with the ASTM document Standard Guide for Examination of Handwritten Items which lists steps that forensic document examiners follow when examining and comparing bodies of writing. Hereinafter, this will be referred to as the standard procedure, as it represents the knowledge engineering necessary for an expert system. For the validation purpose, the standard procedure has been vetted and accepted by the FDE community. When following the standard procedure, the examiner often needs to make several decisions, since every case has special needs, e.g., ransom notes could be written by multiple writers, thus requiring comparison of document sub-parts; with historical manuscripts; different writers may be more similar to each other than with contemporary writers; thus requiring recalibration of individualizing characteristics; and, there is always the potential for disguised writing. The standard procedure will be described and steps will be annotated where existing and future computational tools are useful. The well-known Lindbergh ransom note case was chosen as it is familiar to the forensic community, and illustrates the range of problems to be considered, including extended writing, addressed envelopes, disguise, poor quality images, writer training, and, finally, expression of an opinion.

The CEDAR-FOX system is an interactive tool for FDEs which assists in performing several steps of the standard procedure. First the questioned and known documents are scanned, then several interactive tools are available for preparing the document for processing, which would assist in isolating particular words, reducing image noise, and removing unwanted artifacts. The standard FDE procedure for handwriting can then be cast in computational terms, and used to systematize and validate expert human procedures. Automation tools are available to perform many of the steps. The findings for the particular ransom note case using the tools are given. Observations are made for developing a more fully automated approach to FDE.

Handwriting, Automation, Computer

J8 Judicial Decision Making on the Admissibility of Forensic Document Examination Testimony: A Twenty-Year Review

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After attending this presentation, attendees will have an understanding of how judges have treated challenges to proffers of expert testimony in which the admissibility of forensic document examination during the twenty years following the U.S. Supreme Court decisions *Daubert vs. Merrell Dow Pharmaceuticals, Inc. (509 U.S. 580, 1992), General Electric Co. vs. Joiner (522 U.S. 136, 1997),* and *Kumho Tire Co., Ltd. vs. Carmichael (199 S.Ct. 1167, 1999)*. Attendees will gain an understanding of how judges have interpreted the requirements of these decisions and their impact on the field of forensic document examination as described by fellow document examiners.

This presentation will impact the forensice science community by offering an understanding of how judges evaluate the admissibility proffers of forensic document examination under the guidelines established by *Daubert, Joiner, Kumho*, Federal Rule of Evidence 702, and subsequent case precedents.

Judges' interpretations of their gatekeeping responsibilities under the *Daubert* trilogy have imposed more objective, stringent requirements (relevancy, legal sufficiency, and reliability) for the admissibility of some kinds of evidence which for 70 years had been considered admissible under the *Frye* decision's general acceptance standard, while other

kinds of evidence have remained relatively unaffected by the *Daubert* trilogy. Confronted with challenges to the admissibility of evidence from their various fields, forensic practitioners have responded to the questions about the reliability of their testimony by seeking ways to both improve their disciplines and demonstrate to judges, attorneys, academicians, and fellow experts that their underlying assumptions, methods, and conclusions meet the requirements of the *Daubert* trilogy. This discourse among practitioners, judges, attorneys, law professors, and evidence scholars about how the admissibility of expert testimony from the forensic fields should be determined illustrates an issue relevant to all expert testimony.[1]

A summary of findings from an empirical content analysis of published judicial decisions concerning cases in which forensic document evidence was challenged following the 1993 *Daubert* decision will be presented. The purpose of this study of case law was to empirically examine patterns of cases and the variety of factors that judges discuss when describing the reasons for their admissibility decisions.

Criminal and civil cases containing codeable proffers in which the admissibility of forensic document examination was challenged were identified. The forensic document examination proffers were divided into two groups according to whether the case was decided before or after the *Kumho* decision, investigated whether there were any differences in judges' discussions of admissibility in terms of various rules of evidence. Significant differences pre- and post-*Kumho* in the number of mentions of the reliability of the basis of the testimony, the reliability of the principle or method upon which the evidence was based, falsifiability, error rate, and peer review and publication were found.

Bivariate correlations revealed significant relationships between the number of evidence characteristics mentioned by judges and the length of time post-*Daubert* that the decision was handed down. The number of evidence characteristics judges discussed increased as the length of time post-*Daubert* increased (a significant positive correlation).

The data available in this sample suggest that judges differentially focus on characteristics of the experts and the evidence depending on the type of case. Judges in civil cases who discussed forensic document evidence discussed a greater number of both expert characteristics and evidence characteristics than judges in criminal cases. The impact of *Daubert* on the field of forensic document examination from the point of view of two experts, and the steps which have been taken by forensic document examiners to meet the requirements of the *Daubert* trilogy will be discussed. The empirical data and the discussion by forensic professionals in the context of the sociology of science, and a discusstion of how the tenets of this sociological perspective are demonstrated in the discourse surrounding the social construction of evidentiary reliability and the admissibility of forensic expert testimony is examined.

Reference:

1. Merlino, M.L., Springer, V., Kelly, J.S., Hammond, D., Sahota, E., & Haines, L. (2008). Meeting the challenges of the *Daubert* trilogy: Refining and redefining the reliability of forensic evidence. *Tulsa Law Review, 43(2), 417-445.*

Document Examination, Admissibility, Judicial Decisions

J9 U.S. Questioned Document Examiners: A Nationwide Survey of Background, Education, Training, and Experience

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After attending this presentation, attendees will become knowledgeable of the typical background that is characteristic of forensic document examiners currently practicing in the U.S., including educational attainment, training, certification, experience, and other aspects of their professional preparation.

This presentation will impact the forensic science community by presenting a snapshot of background training, experience, and qualifications of forensic document examiners that are currently practicing in the U.S. This includes their perception of the strengths and weaknesses in training, based on their response to open-ended questions presented in the survey. This is informative for those still in training and helpful for those who offer continuing education or training courses for document examiners.

This presentation presents the findings from Phase I of a national study of forensic document examiners, sponsored by the National Institute of Justice (NIJ). The purpose of the study is to inform and expand the extant empirical research on forensic document examiner expertise in signature analysis. In Phase I, Forensic Document Examiners (FDE) from across the United States participated in a multi-mode (phone and web) survey to gather background information from the professional examiners and their opinions regarding the strengths and weaknesses of education and training in forensic document examination. This presentation presents a summary of the survey results (e.g., background and education) including a thematic analysis of their views on training programs.

The sampling frame was derived from the contact information on file with various regional and national professional organizations in which participant FDEs are members. Examiners who participated in this study were currently employed in the United States, over the age of 18, and English-speaking. Potential participants received an advanced letter describing the purpose of the study at the mailing address on file with the professional organization. Two weeks after they received an advanced letter, phone interviewers at the Center for Research Design and Analysis at the University of Nevada, Reno (UNR), in collaboration with researchers at Kentucky State University (KSU), contacted potential respondents to participate in the phone survey or web survey if they preferred.

The survey probed examiner background, including their educational attainment, membership in professional organizations, certifications, training, and other professional preparation. The survey also documented the type of lab in which FDEs are employed, as well as other positions that examiners have held (including specializations outside of forensic document examination). The survey also included questions regarding examiner experience in providing expert testimony and engaging in proficiency testing as part of their training. The survey concluded with open-ended questions regarding the perceived strengths and weaknesses of examiner training. Themes from these open-ended responses are summarized and presented in this poster.

The results of this survey will inform Phase II, the experimental portion of the study, which is currently in the ongoing data collection phase at KSU. Phase II involves the examination of various handwriting samples in questioned documents by both forensic document examiners and lay participants. The background information of examiners from the Phase I survey data is informative in and of itself as an indication of the contemporary background, education, and experience of forensic document examiners who are currently practicing in the United States. It will also serve as a fundamental set of statistical controls for differences in examiner backgrounds, as well as a platform for analyzing patterns in individual differences between examiners and their performance in Phase II at KSU.

Questioned Document, QDE Training, QDE Education

J10 Statistical Basis to Determine Probabilities of Occurrence of Handwriting Characteristics

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After attending this presentation, attendees will be informed of preliminary results of computerized assessment of specific handwriting characteristics and their frequency in a general population database.

This presentation will impact the forensic science community by providing an example of frequency determination of handwriting analysis that may be applicable to other kinds of pattern analyses that are observer dependent with subjective conclusions.

This research proposes to supplement the existing research conducted in the publication, *A Statistical Examination of Selected Handwriting Characteristics*.[1] That research involved the statistical analysis of specific handwriting characteristics for the "th" combination. This and other previous studies focused on the "th" combination because of its frequency of use in the English language, as well as its role in the most commonly used English word, "the." Based on a survey by www.AskOxford.com, the next most common English words are "of" and "and." The current study will seek to examine handwritten characteristics of the selected word "and" in both its hand printed and handwritten (cursive) form, as well as occurrences of the symbol for "and" (e.g., ampersand). The intent of this research is to determine the feasibility of studying aspects of the selected word, and quantify observed characteristics into statistically useful information.

When Forensic Document Examiners (FDE) render conclusions based on their comparison and examination of handwritten items, they tend to assign probative values to specific handwriting characteristics and their combinations. Their judgments are usually based entirely on the examiners experience and power of recall. Statistical data concerning frequency of occurrence of characteristics and their combinations offer promise for providing a quantitative basis for forensic document examiner conclusions. If the frequency of occurrence of combinations of particular handwriting characteristics is available, then the probability of the observed characteristics can be determined. Such objective information would not only support the examiner's conclusions, but would provide additional scientific credence in courtroom testimony.

In the present research, a computer-executable "truthing" tool was developed to more easily allow the consulting FDEs to evaluate each instance of the written word "and," then select specific characteristics observed from drop-down menus. The "truthing" tool works in conjunction with images of handwriting and hand printing specimens in a database consisting of supervised handwritten and hand printed specimens from over 1,500 individuals representative of the United States population. Examples of observable characteristics are whether the "a" staff is: (1) retraced; (2) looped; (3) a single line; or, (4) no fixed pattern. These characteristics and their most frequent combinations will then be used to determine their frequencies in the database. Since the number of combinations can be very large, a probabilistic graphical model is used to calculate the probability of any given combination will be determined. The probabilistic models are then used to infer the desired probabilities. The number of characteristics is necessarily limited in order to establish preliminary frequency characteristics without requiring an overwhelming amount of labor-intensive effort from the consulting forensic document examiners. The resulting methods will be incorporated into a software system to aid the forensic document examiner. The database and selected characteristics can be expanded for future research projects that may focus on additional characteristics of the word "and" or other words and their selected characteristics.

Reference:

1. Muehlberger, R.J., et. al., *Journal of Forensic Science*, 1976

Handwriting, Frequency, Computer

J11 Exhibit Design and Presentation

Suji Lee, BFA, and Barbara Torres*, 1800 Paseo Rancho Castilla, Los Angeles, CA 90032*

After attending this presentation, attendees will understand how a well thought-out visual account of an expert's results can best be used in case reports and courtroom exhibits to make effective visual exhibits that aid verbal communication.

This presentation will impact the forensic science community by reviewing how forensic document examiners can use the various computer software options and tools to their best advantage and effectively present complex information in a clear and concise manner.

As experts, we are expected to simplify complicated questioned document examinations. Understanding the perspective of the jury and having a basic knowledge of color theory, composition, and content selection will aid the expert in the communication of his or her findings.

When testifying before a jury in today's crime drama obsessed society, the use of exhibits is not only beneficial, but expected. It has been expressed by attorneys that the most effective expert witnesses are excellent teachers. As such, visual aids are critical to demonstrating evidence and findings. This presentation will share the research behind what juries respond to and expect from experts in this regard. The advantages and disadvantages inherent in the various presentation options available will be discussed such as the static poster format vs. a slideshow presentation (software format) or individual handouts for the jurors vs. one central poster.

Exhibits can support the expert's testimony and make it more impactful and memorable. For decades, forensic document examiners have used photographs, photocopies, or drawings to demonstrate handwriting and other evidence. The digital age has ushered in a variety of software programs with capabilities that far exceed the simple formats of the past.

Many elements compose the basics of design such as: visual hierarchy, positive and negative space, font choices, annotations, and color influences. Software programs provide forensic document examiners with many design options in these areas. Some of these elements overlap. Visual hierarchy includes the use of emphasizing scale, colors, font style, placement, and opacity. Positive space is the subject matter area and negative space is the remaining surrounding areas. Knowing how to compose the content/subject matter on a blank canvas will make a difference in keeping the jurors' attention and their eyes inside the frame of your exhibit. Font choices are pertinent for legibility and speed in which the jurors can read the information without hesitation. When the font is difficult to read, this can deter the jury members from reading on further. It is common that forensic document examiners create annotations on their exhibits. These annotations may be made with multiple colors that can potentially lead to confusion in the courtroom. Organizing notations and choosing the right colors can make an inviting environment for the viewer. Therefore, jury members will spend less time trying to comprehend what the annotations are conveying and focus more on what is important. Having a better understanding of these topics will facilitate a foundation for a straightforward and powerful chart that best demonstrates your evidence.

Exhibit, Design, Testimony

J12 The Wills of Michael Renslow

David S. Moore, MEd, 9010 Barrhill Way, Fair Oaks, CA 95628*

After attending this presentation, attendees will gain an understanding of the various types of forensic document examinations that were relevant during the evaluation of the hand-printed holographic wills of Michael Renslow. Each area of consideration provides critical evidence that led to the ultimate conclusion of the wills authenticity. The various considerations in this case example are relevant to the examinations of many similar questioned documents that are challenged in legal proceedings.

This presentation will impact the forensic science community by providing overwhelming evidence that addressed the issue of genuineness that existed between the contested wills. This evidence will be presented in a logical, cogent sequence within a framework and understanding that the author, who was trained in a formal, conventional manner consistant with ASTM standards, was opposed by two, less formally trained individuals who had arrived at diametrically opposite findings.

This case example involves the various holographic hand printed wills of Michael Renslow and the various forensic document examinations that were conducted to determine the authenticity of these wills.

In this matter, the author was opposed by two examiners who arrived at findings totally opposite from the author. When such a condition occurs, it is a legitimate endeavor to consider why this happened and whether formal training — or the lack of it — might be the underlying cause. The author was formally trained as a forensic document examiner in the mid-1970s in accordance with the requirements outlined in 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 training of the opposing examiners did not meet the requirements of this ASTM Standard (indeed, one of them was originally a graphologist and was primarily self-trained in forensic document examination). Additionally, the author has been certified by the American Board of Forensic Document Examiners (ABFDE) since it's inception in 1978; while one of the opposing examiners was certified by the American Board of Forensic Examiners (ABFE). Although both Boards have received accreditation by the Forensic Specialty Accreditation Board (FSAB), only ABFDE was initially sponsored by the American Academy of Forensic Sciences (AAFS) and other national and international prestigious forensic document examiner organizations. In many ways, this case is a perfect example why formal training should be a prerequisite for individuals who claim expertise in this field.

A total of three wills were purportedly written and signed by Mr. Renslow over a two-month time span in latter 2010. Each will bequeathed all of Mr. Renslow's money, property, and other valuable assets to his girlfriend/fiancé of eight years, Ms. Susan Pinar Ilkin. Mr. Renslow's relatives, who were not mentioned in the wills, challenged the authenticity of the wills and retained an attorney to represent them. In turn, the author was retained by that attorney. The opposing examiners were retained on behalf of Ms. Pinar Ilkin.

This hand printing and signature case involves considerations such as disguise, simulation, tracing, alterations, erasures, indentations evidence, erasable ink and pencil writing, multiple authors, and other important aspects that, ultimately, led to a determination of the authenticity of the wills. Often, holographic will contests involve only several of these considerations; however, 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.

Each of the relevant aspects that were considered and explored in the examination of these wills will be discussed. While each of these aspects directly addressed the issue of the wills' authenticity, the opposing examiners apparently either did not consider these various aspects or they discounted them.

Holographic wills, to be considered authentic by the court, must be written and signed in their entirety by the decedent. Critical to the evaluation of the authenticity of such wills is the body of known writings. A sufficient quantity of comparable genuine (known) handwriting, hand printing and signatures is a pre-requisite for such an evaluation. In this case, a significant quantity of the "known" writings was provided by the proponent of the wills, Ms. Pinar Ilkin. Section 7.10 of ASTM Standard E 2290 (*Standard Guide for Examination of Handwritten Items*) requires, among other things, that the known writing must be inter-compared to assure that it was all written by one individual. Had this guide been considered and appropriately employed by the opposing examiners, they would have discovered that the known documents contained the writing of more than one individual. Had such a discovery been made, presumably their findings would have been different.

Finally, this presentation will consider that possibility that, if Michael Renslow didn't write and sign his purported holographic wills, then who did?

Holographic Will, Forgery, Simulation

J13 Examination of Text on Carbon Paper

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After attending this presentation, attendees will learn about methods used to decipher text on sheets of carbon paper.

This presentation will impact the forensic science community by familiarizing attendees with a questioned document case involving carbon paper and the methods used to decipher text on the carbon paper. These methods might be applicable to other questioned document cases involving carbon paper.

This presentation will document a questioned document case study of a carbon paper examination and the steps applied to decipher and report the text content on sheets of carbon paper. Sheets of carbon paper were submitted for examination to the Homeland Security Investigations (HSI) forensic laboratory as part of a criminal case. The sheets of carbon paper had been used to make copies of, and distribute, a secret newsletter to members of a prison gang. The carbon paper was a key piece of evidence in the case against one of the defendants. Decipherment of the text on the carbon paper was important in order to show the information contained in the newsletter.

The carbon paper submitted had been used to make typewritten copies of a few different documents. The carbon paper had been well handled and contained hard-to-read typewritten and handwritten text. The sheets of carbon paper were filled with lines of text prepared using both the English and Spanish languages. Some of the sheets of carbon paper had been used to copy more than one document. Lines of typewritten text were typed overtop of other lines of typewritten text. Notations had been handwritten overtop of the typewritten text and overtop of other handwritten notations. The overprinted text on the carbon paper was not clearly observed. There was some success in deciphering the overprinted text through image subtraction and analysis techniques performed using the Foster & Freeman VSC® (Video Spectral Comparator 6000) and Adobe Photoshop® (version CS5). This was accomplished by collectively examining the sheets of carbon paper using the VSC and Photoshop. This resulted in the decipherment of almost all of the text on one of the sheets of carbon paper. Image processing resulted in the decipherment of additional text on the carbon paper that would not have been deciphered otherwise, helped to verify the accuracy of hard-to-read text, and enhanced the readability of the text.

Another aspect of the case was centered around whether it was a decipherment or a transcription. Discussions between questioned document examiners revealed that some viewed the carbon paper examination as a transcription, while others viewed it as a decipherment. Additional discussions arose on how to report the results. Some examiners favored presenting the results to the case submitter as images for the case submitter to interpret. Others favored presenting the interpreted results to the case submitter in the form of a transcription of the text. In addition, other questions arose during the review process, after seeing whether a second examiner could independently reach the same conclusion. The case ended up as a decipherment. A transcription of the deciphered text was provided to the case submitter. A second examiner independently reached the same conclusion for the majority of the deciphered text. The text was reported as unable to decipher where no agreement between the two examiners could be reached.

Questioned Documents, Carbon Paper, Decipherment

J14 Characterization and Differentiation of Document Papers Based on Element Profiles

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After attending this presentation, attendees will become familiar with the use of element profiles as a means to characterize document papers, as well as the use of statistical procedures to differentiate the papers based on these element profiles.

This presentation will impact the forensic science community by demonstrating an additional method for questioned document comparisons. The elemental profiles potentially offer greater discriminating power than the current methods used, and statistical analysis of the profiles provides a more objective method of comparison.

Currently, the analysis of document papers typically involves a comparison of physical and chemical features such as dimensions, color, and brightness. However, with advances and improvements in the paper-making process, differentiation based solely on these features can be limited. As a result, alternative methods that exploit different properties of the paper would be beneficial in the analysis and comparison of document papers. Recently, the potential of trace elements for the differentiation of paper samples has been investigated. These elements originate in the paper as a result of impurities in the raw materials, as well as from processes used during manufacture. Thus, it is possible that a profile of the elements present may be used to not only differentiate paper types, but also to associate paper of the same type produced by the same manufacturer.

Since the elements are present at trace levels, sensitive instrumental techniques are necessary for the analysis. Previous research has used inductively coupled plasma mass spectrometry (ICP-MS) to analyze trace elements in paper samples. This multi-element technique meets the requirement for high sensitivity; however, the instrumentation is complex and high running costs are associated with the analysis. An alternative technique is inductively coupled plasma optical emission spectroscopy (ICP-OES), which is a less expensive multi-element technique. While not as sensitive as ICP-MS for many elements, ICP-OES may offer a viable alternative for element determination in document papers.

The purpose of this research was to further investigate the differentiation of document papers based on the trace element profiles generated using ICP-OES. Three reams of four different types of paper (copy, laser inkjet, multipurpose, and office paper) produced by the same manufacturer were obtained. Samples were microwave digested in nitric acid and hydrogen peroxide prior to analysis. The resulting digests were analyzed by ICP-OES to identify characteristic elements for each paper type. The elements selected were those that were present above the instrument detection limits, were not present at significant levels in the blank digest, and did not vary significantly within a ream of paper. The selected elements were then quantified using ICP-OES. The sample digests were also analyzed by ICP-MS, quantifying the same elements.

The element concentrations determined were firstly normalized to the initial mass of paper used in the digestion. The element profiles that were generated using each instrument were treated as two separate data sets and each data set was firstly subjected to hierarchical cluster analysis (HCA). This is a multivariate statistical procedure used to identify similarities among samples in a data set. The dendrograms generated were used to assess similarity among reams of the same paper type, as well as dissimilarity of different paper types, based on the concentrations of the elements present.

Profiles in each data set were also subjected to principal components analysis (PCA). While this procedure is also multivariate in nature, PCA identifies sources of variance in a data set, rather than similarities, as is the case in HCA. The two main outputs of PCA are scores plots and loadings plots. The former plots are scatter plots in which chemically similar samples are positioned closely and distinctly from chemically different samples. Loadings plots are used to identify the variables contributing to the variance described by the principal components. In this research, the scores plots were used to assess the association of reams of paper of the same type and differentiation of different paper types. The loadings plots were then used to identify the elements responsible for the differentiation observed.

Finally, results from HCA and PCA were compared to determine if one technique, ICP-OES or ICP-MS, offered improved association and differentiation of the paper samples based on the elemental profiles generated.

Trace Elements, Questioned Documents, Statistics

J15 Analysis of Eye Movements While Observing Handwriting

Yoko Seki, MA, National Research Institute of Police Science, 6-3-1, Kashiwanoha, Kashiwa, JAPAN*

After attending this presentation, attendees will understand: (1) the strategies that a forensic document examiner takes to extract characteristics from handwriting by visual inspection; and, (2) the importance of the observation from various perspectives in handwriting examination.

This presentation will impact the forensic science community by demonstrating the relationship between the examiner's eye movements and the correctness of the identification results.

Eight forensic document examiners participated in the experiment. They were instructed to watch the monitor displaying two handwriting samples, one of which was the questioned handwriting and the other was the known handwriting, and decide whether two samples were written by the same person or not. Sixteen writers participated in the preparation of handwriting samples. They were asked to write the same person's name in square style. One out of sixteen writers was asked to prepare five samples, two of which were used for the known handwriting and other three were used for the questioned handwriting. Another fifteen writers were asked to prepare one sample, which were used for the questioned handwriting. The handwriting pair, used as a stimulus, consisted of a known handwriting and a questioned handwriting. So, there were eighteen handwriting pairs used as stimuli. Three out of eighteen pairs were the same writer's pair and the other fifteen were different writers' pair. Procedure of the experiment was as follows: known handwriting pair was firstly presented to the subject for extracting characteristics of the known handwriting; then 18 stimuli were presented to the subject respectively in random order; subjects were instructed to click the mouse to display a stimulus, observe it as long as necessary to identify the writer, and click the mouse to express their opinion as to the identity of the writer; and, eye movements of the subjects during the observation and the time necessary to their decision making were measured. An eye-tracking system was used for the measurement.

Answers obtained from the subjects were either correctly identified or inconclusive. Average ratio of inconclusive was higher in the case of the different writer than the same writer. Average time necessary to the decision making was significantly longer in the case of inconclusive than conclusive. Observing manner was roughly common to the subjects, that is, subjects observed some point of the first character of the known handwriting and then observed the corresponding point of the questioned handwriting. Then, observation point moved to the next point of the known handwriting and then the corresponding point of the questioned handwriting. The same procedure was taken throughout the entire observation. All the subjects observed almost the same points in the case where the stimulus was undertaken; short response time and unanimous answer. All the subjects observed characteristics on shape such as terminating manner of a stroke, curvature, and relation between two strokes. One subject observed the space between characters along with the shape and all his answers were correct.

The fact that longer time was necessary to the decision making in inconclusive cases suggests that the distinct difference or similarity between the two handwritings makes the decision making easier. This

also suggests that there is a possibility of the commonality of the document examiner's knowledge on the typical or large intra-individual difference. Higher inconclusive ratio in different writers than the same writer was, however, different from the expectation because the number of the same writer samples was smaller than that of the different writer samples. This may suggest that the examiner unconsciously avoids type 1 error. The fact that the observation on the spacing had an influence on getting the correct answer suggests the necessity of various viewpoints in the identification task for reaching the correct opinion. These results show the importance of the inspection from various perspectives, the choice, and the processing of the adequate information in the handwriting examination.

Handwriting, Eye Movements, Eye-Tracking System

J16 Preliminary Trends in Frequency Occurrence of Handwriting and Hand Printing Characteristics

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After attending this presentation, attendees will have an understanding of how the results of this project will benefit their daily work and court presentations. The objective of this presentation is to provide a pilot preview of early results of a frequency occurrence study. In addition, attendees will receive a brief tutorial of the use of the database system used for characteristics classification.

This presentation will impact the forensic science community by putting the judiciary and critic communities on notice as to the advancements of strengthening the statistical basis of handwriting conclusions used in court.

In 2010, the National Institute of Justice (NIJ) awarded a research grant for the study of the frequency occurrence of selected handwriting and hand printing characteristics. This study is a statistic-driven project requiring exacting methodological precision that will withstand an expected onslaught of criticism from those who have created a cottage industry out of such attacks.

The NIJ study is designed to classify approximately 2,000 handwriting or hand printing characteristics of approximately 5,000 writers. The population sampling is being collected using a stratified sampling representative of the population within the United States and taking into account instrinsic and extrinsic factors that may affect handwriting as described in *Handwriting Identification: Facts and Fundamentals* by Huber and Headrick.[1] Factors considered include age, ethnicity, sex, handedness, region of early elementary education, and location of collection. The percentages of each factor must fall within a range (such as plus or minus five percentage points) of what is recognized as the makeup of the United States population. For example, if twelve percent of the United States population is left-handed, then the stratified sampling must be within a range of seven to seventeen percent.

The handwriting specimens form content is a modified version of the specimen form used by Dr. Sargur Srihari in earlier published studies of handwriting and pattern recognition.

The handwriting characteristics have been selected and tested for objectivity and repetitiveness of results in order to avoid unnecessary integral error rate increase. Each feature has undergone, among other tests, an Attribute Agreement Assessment study in which multiple examiners have classified the same handwriting forms and have themselves classified the same handwriting forms more than once. Characteristics that are found to bear variances of results, either from the multiple forensic document examiners or by the same examiner in two different classification exercises, is either modified to eliminate the issue causing the confusion or has been excluded from the study as being unnecessarily subjective.

A database system has been specially developed for this project. Every feature bears a brief description with most accompanied by an image illustration. By design, a classifier need only click their computer mouse in the check box in order to note the presence of a denoted feature. The database will automatically record the result. Multiple handwriting specimens can be recorded on a database and the results will then be sent to the database specialist who will integrate all databases and submit the results to the statisticians for analysis. It should be noted here that the forensic document examiners have no part in this portion of the study and this is by design. The project is a statistical study and is driven by statistical methodologies. Any decisions as to procedures in this study are the sole responsibility of the statisticians.

Trained forensic document examiners are used for the purposes of classification in the database. The reason for this is that it will be forensic document examiners who will be the end users of the project as they will be able to enter features from writings in question in actual casework and collect frequency results to their case-specific entries. As part of the presentation, attendees will be guided through a tutorial on the use of the database as volunteer classifiers are an essential need of this project and will be solicited from amongst the attendees who are practicing forensic document examiners with documented training meeting the ASTM training guideline.

As part of the pilot projects, the statisticians have arranged for a pilot study of a small number of writers in order to test the complete system for seamless integration. Since the pilot study will also yield actual results, it is the purpose of this presentation to present the results along with presenting idiosyncrasies that have thus far been observed during the study.

This research was funded by the National Institute of Justice (NIJ) research grant (2010-DN-BX-K273).

Reference:

1. *Handwriting Identification: Facts and Fundamentals*, Roy A. Hueburn and A.M. Headrick (1999), CRC Press, Boca Raton, FL, ISBN O-8493-12895-X

Statistics, Handwriting, Frequency

J17 Some Reasons for Qualified Conclusions

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The goal of this presentation is to provide some reasons why a forensic document examiner cannot always reach a definite conclusion in a handwriting or hand printing examination.

This presentation will impact the forensic science community by explaining why handwriting and hand printing evidence is not always conclusive when attempting to identify a writer.

Frequently, recipients of reports from Forensic Document Examiners (FDE) expect the FDE to be able to determine with absolute certainty the authorship of handwriting and hand printing material they are examining. Under no circumstances is this possible. Every FDE is limited by the evidence contained in the writings they are examining.

The evidence in the writings may be the result of a lack of sufficient individuality in the writing, a limited quantity of writing, copies of documents or writing that is not original, or any number of other factors. This paper discusses some of the reasons for qualified conclusions and how different factors can affect the certainty of the conclusion reached.

Identification or elimination of a writer is based on the cumulative effect of all the available observable evidence in the writing examined and not on the presence or absence of any single characteristic, quality, or feature in that writing. The identification or elimination of a writer requires conclusive supporting evidence.

The presence of a single, significant, or irreconcilable difference does not provide a basis for concluding that two writings are by different writers. It does provide a basis for non-identity of the writer whose known writing is compared with the questioned writing. Non-identity is not the same as eliminating a writer. Additionally, the presence of what is thought to be a single, significant similarity does not provide a basis for concluding that two writings are by the same writer.

The paper includes examples of writers that have more than one style of writing. These writers are able to write without incorporating features of one style into features of the other style. The writer may be able to do this using their unaccustomed hand, while some writers can do it using the same hand. Although style is one factor, the details of the writing of the different styles can be completely different.

Another factor discussed in the paper is the limitation imposed by the adequacy of the submitted writing. The submitted writing may be insufficient for comparison purposes. A writing sample can be insufficient due to disguise, a lack of sufficient individuality or limited writing, evidence of simulation or tracing, completely different styles of writing submitted for comparison, etc.

Some transitory factors that can result in qualified conclusions are also discussed. Transitory factors can include, but are not limited to, injury to the writing hand, use of drugs, other temporary physical conditions, etc. One transitory factor that is discussed is referred to as an accidental feature resulting from a single event that temporally affected the writer.

All copies of writing are problematic. If only a copy is available and the original has purportedly been destroyed, it is not always possible to say with certainty that the copied writing is an accurate reproduction of the original writing. Additionally, it is not possible to conclude, based on the examination of the copy alone, that the writing on the copy is actually written on the original document. Because of the increasing reliance on electronic documents used by banks, government offices, corporations, etc., originals are frequently destroyed. Many times the FDE is working from a printout of a scanned document that has the word "original" stamped on it. Such documents are not "originals" as defined by the FDE.

In summary, it is not possible to reach an unqualified conclusion in every case the FDE examines. Various factors in the writings combine to limit the significance that can be attached to the characteristics, qualities, and features of the examined writing to support a conclusion that it is or is not written by the same writer.

Handwriting, Qualified Conclusion, Evidence Evaluation

J18 Decision Making When Dealing With Blood Soaked Documents

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The goal of this presentation is to present for discussion the decision-making process involved in the forensic document examinations of blood soaked documents.

This presentation will impact the forensic science community by reinforcing the knowledge that critical thinking is necessary in dealing with blood soaked documents, particularly in how those decisions may affect the presentation of the documents within the judicial system.

Death investigations may involve document evidence which is obscured by products of decay, blood and bodily fluids, and other environmental substrates. The forensic document examiner (FDE) is faced with a decision-making process which must take into consideration the items which comprise the documents, and what role these items may play in the future of the case at hand, biological safety factors, and the limitations of the laboratory equipment available. Investigators who submit items to a crime laboratory are asked only for brief information about the items and requested examinations. When dealing with deteriorating documents, more information is necessary, and typically involves discussions with the investigator. The final product of the FDE work will likely be hardcopy prints made from digital images from cameras, video images, or scanners. The use of these products in a courtroom, or in discussions with those involved in the investigation, may be a factor in deciding what examinations will be conducted. Blood and body fluids present a potential safety risk, but, their removal allows for future viewing and presentation of the original exhibits. A complicating factor is that removal of contaminants may be deleterious to the items which comprise the exhibits. It is therefore necessary to gather as much information about the documents as is feasible before proceeding with examinations beyond nondestructive imaging.

The case presented for discussion involved a two-page document which was handwritten, and for which no pen was available as the likely source of the ink. The color of the ink was important. The documents were heavily stained with body fluids which obscured roughly two-thirds of the information. Infrared imaging yielded good results, but not the critical words and phrases that mattered to the investigator and the decedent's family. Microscopic examinations provided only limited information about the pen ink. A careful evaluation of the effects of the body fluids allowed decisions to be made about subsequent limited testing of the ink-blood-paper composite. Limited testing and infrared imaging followed, and allowed for a decision to remove the blood and still retain readable original documents. The resultant images and documents allowed for every word on the two pages to be read. Images of the original documents with stains, accurately reproducing the color of the pen ink, were retained in file. Images of the documents as the examinations proceeded were also kept in file. Black and white composite infrared fluorescence images, and grayscale images of the resultant documents, were provided to the investigator for his information and for discussions with the decedent's family.

Document Examination, Death Investigation, Obscured Writing

J19 The Case of the Staple Mark

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After attending this presentation, attendees will have a better understanding of the importance of literature review when examining unusual cases with limited evidence and providing the court with supporting documentation from the literature along with exhibits.

The presentation will impact the forensic science community by demonstrating the importance of literature from professional source material when testifying in court on an unusual case.

A check for more than one million dollars was sent by a title agency to a finance company. The finance company claimed they never received the check. A staple mark on the check along with testimony from the title agency's employees allegedly would prove the finance company did receive the check. Staple marks are often part of the examination process in cases involving medical records, business records, contracts, and wills. However, it is not common for a forensic document examiner to receive an examination involving only a staple mark. In this case, the original check was destroyed and the forensic document examiner was asked to determine if three marks on copies of the front and back of the check were the result of a single staple mark. There was some concern by the plaintiff's attorney and client that this may not be within the purview of the examiner. To allay the attorney's concerns, a literature review of respected tomes was conducted and several applicable sources were documented.

The examination of the two sides of the questioned check then commenced. Due to the obvious enlargement of the questioned check copies, a request was made to provide an original blank check from the title company to use in the comparison process. Based on the size of the original requested check, the questioned copies were reduced to 87% to match the known check size. Transparencies and copies were made of the 87% copies to assist in the alignment process. Two marks on the reverse side of the questioned check were found to be in close alignment and consistency with a standard ½-inch staple. One small hole was observed on the face of the check. Unfortunately, the copying process of the original check left artifacts that obscured the area on the front of the check where a second hole would be expected. Using a standard stapler with ½-inch staples, staples were placed on copies of the questioned 87% checks in the same locations. The staples were found to be in relative alignment and width as the marks produced on the questioned check. It was determined that it was highly probable the marks on the check were the result of a staple mark; i.e., the examiner was virtually certain the marks on the check were from a staple.

In 2011, upon appearance in court, the defendants' attorney objected to testimony concerning the staple mark, stating that a forensic

document examiner has "no special knowledge" of staple marks. A copy of the chapter written by Tom Vastrick titled "Staples" in the *Scientific Examination of Questioned Documents* was provided to the court.[1] The court accepted the testimony and ruled that the forensic document examiner does have special knowledge regarding staples and/or staple marks. The case was adjudicated successfully for the plaintiff. It should be noted the original check sent to the finance company was cashed by a third party who should not have received the check. The third party spent all the money and there was no avenue to recoup the loss.

Reference:

1. *Scientific Examination of Questioned Documents*, Second Edition (2006), CRC Press

Staple Marks, Questioned Documents, Forensic

J20 Signatures of the Arabic-Speaking Writer in the United States: Are There Class Characteristics?

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The goals of this presentation are to provide forensic document examiners with insight into signature styles used by Arabic-speaking writers of English, and to discuss results of a preliminary study regarding the effect of right-to-left writers who later learn to write English and whether or not there are class characteristics peculiar to this group of writers.

This presentation will impact the forensic science community by providing a preliminary look into the English handwriting of the Arabic-speaking population. It will serve as a catalyst for further study into the handwriting characteristics, and may provide additional criteria for the analysis of handwriting of this population category.

The intent of this presentation is to provide insight into signatures as they are used in Arabic-speaking countries and how Arabic-speaking writers sign their name in the United States. The information gleaned from background research and analyses of requested signatures collected for this study (written by writers whose first language is Arabic) will provide Forensic Document Examiners (FDEs) with additional criteria for the forensic examination of signatures produced by such writers.

As foreign cultures continue to blend into our American and Canadian societies, FDEs are increasingly asked to authenticate signatures of writers whose first language does not use the Roman alphabet. Among these writers, the Arabic-speaking population is well-represented. Since the Arabic alphabet bears no resemblance to English script and is written from right-to-left, FDEs will be introduced to the some of the issues that impact signature analysis of both Arabic and Anglicized versions. Another consideration is that the Arabic custom regarding signatures is not the same as American custom. In keeping with this theme, the author will illustrate customary uses of the signature in Arabic-speaking cultures. Topics include: (1) styles of signatures used in Arabic-speaking cultures; (2) the purposes of different signature styles; and, (3) adaptations of Arabic signatures for use in the United States.

The training of a FDE in the United States and Canada focuses mainly on the habits of native English-speaking writers and does not necessarily cover handwriting traits of foreign writers, such as Arabic-speakers who have learned an alphabet that is entirely new to them. It is the author's hypothesis that characteristics present in Arabic script could affect the English writings and thereby provide additional criteria for establishing authorship of signatures. Hence, another aspect of this study is to identify whether or not there are specific habits (i.e., class characteristics) within the signatures that could be attributed to this category of writer.

The results of this preliminary study were based on the examination and analysis of signatures provided by 100+ writers. Each writer was asked to write the "English" spelling of his or her name along with samples of the signature styles they use in their native country. Next, each writer was requested to sign in the style he or she uses in the United States for: (1) casual communications, such as a note or letter; (2) transactions that involve money, such as a charge card; and, (3) an official document, such as a passport. The signatures gathered for this study were then inter-compared to determine whether or not any class characteristics could be identified.

Results of this study will be presented as to: (1) the number of signature styles per writer; (2) the styles used and for which purposes; (3) the frequency of use of Anglicized signatures; and, (4) the class characteristics noted among study specimens. Observations and tentative conclusions will be presented, along with suggestions for further study on this topic.

It is expected that the results of this study will provide forensic document examiners with a more reliable basis upon which to form an opinion regarding the identification/authentication of signatures written or purported to have been written by Arabic-speaking writers signing documents in the United States.

Handwriting, Arabic Signature, Document Examiner

J21 A Novel Method of Interpreting Evidence When First Attempts Fail

Carolyn Bayer-Broring, MFS, Immigration & Customs Enforce, OI FDL Stop 5116, 8000 Westpark Dr, Ste 325, McLean, VA 20598-5116*

After attending this presentation, attendees will understand a novel method of approaching an examination when the initial attempts at interpretation fail. A case study will be presented highlighting the efforts used.

This presentation will impact the forensic science community by encouraging attendees to think outside the box during examinations.

The Homeland Security Investigations Forensic Laboratory (HSI-FL) is the investigative arm of U.S. Immigration and Customs Enforcement dedicated to the forensic examination of international travel and identity documents. In the course of working a case, a document examiner at the HSI-FL might examine one document or thousands, to include such things as passports, identity cards, driver's licenses, and birth certificates. A wide variety of instruments are available at the HSI-FL, including the Video Spectral Comparator (VSC), X-ray, microscopes, loupes, and light boxes.

During the course of an examination of three foreign passports, it was determined that all three passports had been bio-page-substituted; meaning the biographical data pages of each were not original, but rather were an "overlay," where an adhesive-backed substrate was utilized to place a false page into the booklet. Indications were that the original pages still were present underneath. Two of the three passports were Electronic Passports ("E-passports" as indicated by the ICAO symbol embossed on the front cover), which meant that there should be an electronic chip somewhere in the book, bearing personalized data relating to the carrier of the book.

Under normal circumstances, specialized software in the VSC 6000 can be utilized to interpret the Machine-Readable-Zone (MRZ) appearing on the biographical data page of a passport, and in the instances of E-passports, the information from the MRZ can then be used to "unlock" the electronic chip, revealing the information encoded on the chip. Visual comparison of the MRZ and the resulting chip data can then be made, to determine if the information is the same—which it should be in instances of booklets that have not been tampered with or altered. Ostensibly, if the MRZs of these page-substituted passports could be interpreted, and if the electronic chips were present and operational, the identities of the true bearers should be easy to determine for comparative purposes.

The machine-readable-zones in the two E-passports were not visible on the substituted pages; however, there was reason to believe they should still be present on the original pages underneath. So what to do? One option would have been simply to pull the substituted page off and see what was underneath; however, examiners at the HSI-FL are not allowed to conduct destructive examinations. So that wasn't an option. Use of an X-ray determined that the electronic chips were

present in the books, and they appeared intact and undamaged (chips and antennae are often removed or otherwise damaged during alterations), so finding some method of interpreting the MRZ and opening those electronic chips was important.

Ultimately, strong transmitted light and the spot-illumination function of the VSC were utilized, along with plain old pencil and paper, to hand-interpret the MRZs of the true biographical data pages still present under the substituted pages. After a little trial and error, the right numbers and letters were determined, the data was hand-typed into the VSC and – voila! The MRZs worked. The data was then utilized to "open" the chips and the images and data of the true bearers became readily apparent, further supporting the finding that the booklets had been altered.

Travel Documents, MRZ, E-Passports

J22 A Crosscut Shredded Document Case Made Easier — Predicting Where the Pieces Go

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After attending this presentation, attendees will learn some of the characteristics of crosscut paper shredders and shredder "chad" (the shredded pieces), and more importantly, understand some of the techniques that can facilitate the manual re-assembly of shredded documents.

This presentation will impact the forensic science community by proposing a methodology to make the process of manually reassembling a shredded document easier.

A small bag of crosscut shredded documents was received for reassembly in the hopes of providing evidence in a case of identity theft and the filing of false tax returns. Several of the documents shredded were self-adhesive memos. Some of the notes had apparently been stuck to each other before shredding, and some had been attached to other documents. Due to the random position of the notes, they became shredded in different orientations to the shredder "mouth," which resulted in bits and pieces of the same type of paper in various sizes and shapes.

Little could be found in the literature outlining a practical methodology for reassembling shredded documents. Some of the difficulties encountered in reassembling shredded documents are: (1) many pieces are of the same size and shape; (2) many pieces are very small, deformed and/or stuck together; (3) some pieces are likely to be missing; (4) paper chad are often very delicate and difficult to handle; and most significantly, (5) the examiner typically has no final design to work toward (this problem is akin to attempting to complete many different picture puzzles thrown together on same table).

The hypothesis presented is that it is possible to predict the precise pattern into which a document was shredded. This can be done by first making some rudimentary measurements of the shredded chad. Next, the pattern observed that is formed by the crosscuts on even a small portion of reassembled chad can be extrapolated to predict the overall pattern into which the document was shredded. Several preliminary steps for sorting the chad (based on size, shape, shred direction, border angles) eventually allow for the more rapid location of a desired piece to fit into the pattern.

A methodology for the complete (ideal) reassembly of a shredded document is proposed. This includes, in part, the following steps: (1) sorting chad by color and type of paper, and by markings present; (2) making measurements or comparisons of the chad width, chad length, and the angles formed by the machine-cut edges and the shredded edges; (3) orienting the chad in the shredder direction, (4) creating a template for assembly and a grid for aligning the chad; and, (5) creating a grid of the pattern of the shred over the entire document.

Through the use of the methodology described, at least nineteen documents in the case were able to be either totally or partially re-assembled.

Also presented will be some results of a related research project of examining the chad produced by shredders of several makes and models.

Paper Shredder, Shredded Documents, Document Reconstruction

J23 Cognitive Theoretical Perspectives in Studies of Forensic Document Examination

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After attending this presentation, attendees will understand some of the principles of cognitive psychology, and the use of eye-tracking technology to study attention and feature-matching processes as they relate to decision-making processes in forensic document examination.

This presentation will impact the forensic science community by demonstrating the importance of engaging in theoretically-based, multidisciplinary research to an understanding of the nature of the methodology and expertise in forensic document examination (FDE).

A substantial portion of FDE training is devoted to signature comparisons, handwriting, and hand printing. FDEs seek those features and characteristics which may be characterized as the document's identifying attributes or characteristics. Examiners first determine the presence or absence of features, and then qualitatively assign these features some degree of evidentiary weight in order to reach their decisions. Examiners are trained to look not only for substantial similarities or differences among writing samples, but also for repeated small characteristics which may be sufficient to establish clearly that writings are the work of two individuals even though they may contain a considerable number of general similarities. The number and quality of these features allow FDEs to make assertions about the authorship of the specimen and the extent of their confidence in their decisions.[1]

A substantial body of research addresses the cognitive mechanisms involved in attention and visual search. This paper discusses the application of cognitive theory to understanding the nature of attention, feature extraction and weighting, and decision-making in forensic document examination. Data from a national study of forensic document examiners will be used to illustrate the ways in which cognitive psychology can contribute to an understanding of the decision-making processes of experts in the field compared to lay those of lay people.

Many current theories of attention propose that attention is based on the relationship between a bottom-up, saliency-based attentional system and a top-down, feature-specific selection mechanism. Attention is guided by relational information about the target, or information about how the irrelevant information of a non-target differs from the features of the target. Relational models of visual search demonstrate that visual attention can be guided by attending to specific feature values such as color, size, or intensity, by inhibiting attention to irrelevant features, or by directing attention to how stimuli differ. Relational models place the target in relation to its context, offering more specific (e.g., directional) information about differences. This relational aspect of attention may be influenced by the presentation formats of stimuli.[2]

Tversky pointed out that most stimuli seem to be effectively described by the presence or absence of qualitative features. He and others argued that an object is represented by a set of features or attributes, and that judgments of similarity are achieved through a process of feature matching. Tversky's "Contrast Model" systematizes this "feature" approach, and proposes that similarity depends on the

proportion of features common to the two objects, and also on their unique features. Feature matching occurs by establishing differences in quality or quantity, such as differences in color or size, or the presence or absence of the features upon which the judgment is based, usually in terms of binary variables.[3] This feature-matching process, along with the deployment of attentional resources, is a core process of forensic document examination.

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2. Becker, S. I. (2008a). Can intertrial effects of features and dimensions be explained by a single theory? *Journal of Experimental Psychology: Human Perception and Performance, 34,* 1417–1440.
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Feature Matching, Attention, Handwriting

J24 Validation of Quantitative Measures in the Non-Destructive Differentiation of Black Ballpoint Pen Inks

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After attending this presentation, attendees will understand the use and application of the measurement feature within Adobe® Photoshop® CS5 Extended as applied in the quantitative differentiation of black ballpoint ink samples.

This presentation will impact the forensic science community by demonstrating the validity and reliability of newly introduced objective measures in traditional qualitative non-destructive ink differentiation tasks involving the analysis of black ballpoint inks.

A previous published study conducted at the U.S. Army Criminal Investigation Laboratory demonstrated the use of L*a*b* color mode as a valid and reliable method in the non-destructive differentiation of black ballpoint pen inks. However, the results obtained through the use of L*a*b* color were based on visual observation and qualitative in nature. To extend the utility and discriminatory power of L*a*b* color as a tool in forensic document examination, this study investigates the use of L*a*b* color mode combined with analysis tools available within Adobe® Photoshop® CS5 Extended (APCE) as a new quantitative-based approach for non-destructive ink differentiation.

In the current study, the measurement feature within APCE was utilized via two different collection methods (manual and automated) to obtain the means of the gray values of each inked sample within a set of pen-pair samples previously converted from an RGB image to a processed L*a*b* color mode image. Theoretically, a statistical difference in the gray value (means) between two inks would provide quantitative support that the two inks were from different populations (i.e., different inks). Conversely, if the gray value (means) of two inks were not statistically different then this would provide quantitative support that the two inks were from the same population (i.e., indistinguishable ink(s)).

For the manual method of analysis, which requires the examiner to manually select the area of interest (AOI) within a given sample, 138 black ballpoint ink pen-pair samples were analyzed after converting the RGB images into L*a*b* color ("a" channel) images. These samples consisted of the following pen-pair types: (1) same ink/pens (N=35); (2) close non-match (N=70); and, (3) different ink/pens (N=33). As the names suggest, when the ink samples were produced, they were created either by using the same pen to create a pen-pair sample (control samples) or with different pens/inks. The close non-match samples are samples that are known to have been created using different pens that utilize different ink formulas but were not previously differentiated by optical examination of the resulting L*a*b* color image of the pen-pair sample. Quantitative analysis outperformed the previously conducted qualitative analysis (i.e., visual inspection of L*a*b* color images of the pen-pair samples) of the close non-match samples. However, qualitative analysis outperformed the quantitative analysis of the pen-pair samples written using the same ink. In the analysis of the pen-pair samples created using different inks, both methods were equally successful in correctly differentiating all of the samples tested.

For the automated method of analysis, the AOI within a given sample is selected automatically using a saved action script within APCE. One hundred and twenty-eight black ballpoint ink pen-pair samples were analyzed after converting the RGB images into L*a*b* color ("a" channel) images. These samples consisted of the following pen-pair types: (1) same ink/pens (N=35); (2) close non-match (N=70); and, (3) different ink/pens (N=23). Automated quantitative analysis outperformed the previously conducted qualitative analysis (i.e., visual inspection of L*a*b* color images of the pen-pair samples) of the close non-match samples. However, qualitative analysis outperformed the automated quantitative analysis of the pen-pair samples written using the same ink. In the analysis of the pen-pair samples created using different inks, both methods were equally successful in correctly differentiating all of the samples tested.

Preliminary findings suggest that this new, low-cost and nondestructive method for ink differentiation has a higher discriminatory power than the qualitative analysis of L*a*b* color images involving different and/or close non-match inks. However, in the absence of establishing a minimum gray value threshold, necessary to prevent or reduce Type I errors (i.e., false positives), manual quantitative analysis using the measurement feature within APCE falls short of the higher performance results produced using qualitative analysis of pen-pair samples produced with the same ink formulas.

*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 Department of the Army or the Department of Defense.

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Writing Inks, Digital Imaging, L*a*b Color

J25 Empirical Differentiation and Profiling of Processed Colored Inkjet Inks Using Fourier Transform-Infrared Spectroscopy

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After attending this presentation, attendees will understand the factors which differentiate the processed inkjet inks and the features which are similar in inkjet inks of different printers of same brands and different brands. This presentation will show the significant use of Fourier transform-infrared spectroscopy in the examination of the cases related to inkjet printer.

This presentation will impact the forensic science community by providing information which could be used to create a database and could be applied to actual case work due to very good sensitivity and reproducibility of the technique.

Processed ink from 208 samples was taken from printouts printed by different types of printers manufactured by someof the leading companies. The samples were taken in the form of printouts from printers of different manufacturers and models. The same substrate has been taken for the collection of samples. All the four primary inks are considered. The sample collection includes the printout from printers

containing both cartridges (i.e., refilled and original cartridges). The random sample collection has been done from different local markets of India. Samples were prepared by keeping cyan, magenta, yellow, and black content constant for each sample using a commercial word pallet software. The samples were collected in the form of colored rectangular blocks. A known quantity of section was removed from the surface of the document and ink was extracted from the substrate by using standard extraction procedures. The samples were dissolved in a constant amount of suitable solvent and kept for some constant time. A series of solvents which are suitable to dissolve the inkjet inks was also given. The samples were then scanned by preparing KBr palettes. The IR spectra were recorded using FT-IR spectrophotometer. The significant peaks were selected and identified to indicate the chemical difference in inkjet inks. Based on characteristic absorption bands in IR, the inks were classified into a few distinct groups. The obtained spectras and all the available information could be used to create a database and could be applied to actual case work due to very good sensitivity and reproducibility of the technique. An attempt has been done to give the ink profiling of inkjet printing inks. The technique is destructive in nature but it will provide a great helping held to the forensic community in the examination of cases related to inkjet printer inks.

Inkjet, Absorption, Refilled

J26 Detecting Altered and Counterfeit Travel and Identity Documents by Utilizing State-of-the-Art Instrumentation

Karen Cox, BS, DHS/HSI/FL Stop 5116, 8000 Westpark Dr, Ste 325, McLean, VA 20598-5116*

After attending this presentation, attendees will become aware of the various methods used to detect counterfeit and altered foreign and U.S. travel and identity documents.

This presentation will impact the forensic science community by providing information and awareness of the challenges the Forensic Document Examiner (FDE) encounters in todays casework. With the sophisticated methods used in counterfeiting and altering travel and identity documents, the use of state-of-the-art instrumentation within the laboratory has become vital in the detection of document fraud techniques.

The Homeland Security Investigations Forensic Laboratory (HSI-FL) is the only U.S. crime laboratory specializing in the scientific authentication and research of foreign and domestic travel and identity documents. The HSI-FL provides document and latent print forensics, intelligence, and investigative support services for the Department of Homeland Security and other law enforcement agencies. The presentation will illustrate various counterfeiting methods and alterations detected in the examination of travel and identity documents using state-of-the-art instrumentation at the HSI-FL.

Technology in today's world is ever evolving; computer technology has become extremely sophisticated, and, as we examine questioned documents for authenticity and fraud, it has become readily apparent that the increase in these capabilities has given the casual counterfeiter many new capabilities to produce a higher level fraudulent document. There are various components involved in the examination of a questioned document. Depending on the type of substrate (paper, hybrid paper, and plastic), the appearance and characteristics of the printing process can be greatly affected. In addition, security features are constantly advancing in the area of questioned documents. The traditional printed security features used in identity cards and passport pages are being supplemented with much more sophisticated secure printing techniques, i.e., iridescent and non-iridescent inks. Security printing incorporates multiple levels of security features such as latent images, guilloche patterns, and rainbow fountain printing. Further, covert security features, i.e., microscopic metallic particles, microstructures, and optically variable devices which contain data, are being incorporated within many security inks. With the use of sophisticated instrumentation, high-quality counterfeits can be detected.

The HSI-FL analyzes travel and identity documents from all over the world; therefore, the range of quality of counterfeiting seen in casework varies. Grommets used in passports for the prevention of photograph substitution have been replaced with more secure and tamper-resistant security measures incorporating holographic laminates, digital encoding, laser engraved tactile features, and embossing techniques. In an effort to increase the efficiency of passenger processing, an increase in the use of machine-readable travel documents is much more prevalent. The use of radio frequency identification devices within travel and identity documents now requires the use of X-ray technology to determine the presence of these devices and to determine attempts of alteration.

Keeping up with advances in document security technology and maintaining a heightened level of awareness of counterfeiting techniques and production methods used by document vendors is crucial in identifying and deterring document fraud.

Counterfeit, Altered, Documents

J27 An Evolution of Document Security: A Case Study of the United States Permanent Resident Card

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After attending this presentation, attendees will gain an understanding of the evolution of the United States Permanent Resident Card to include the advancement of its security features. Furthermore, this presentation will educate attendees on the significance behind the reform of these security features over the last half century.

This presentation will impact the forensic science community by cultivating awareness of how an identity document and its security features evolve as technology continues to advance. The opportunity for most laboratories to study these advancements may be infrequent; therefore, this presentation will provide visual examples and methodologies for examinations leveraging a wide array of tools.

The U.S. Permanent Resident Card, informally known as a "Green Card," designates the permanent resident status of a non-U.S. citizen authorized to live and work within the United States. It is known as a "Green Card" because of the green background printing used in previous designs. The Permanent Resident Card is commonly encountered as a means to obtain a myriad of benefits such as social services, driver's license or identification cards, school registration, and bank accounts.

There are multiple ways in which to acquire a U.S. Permanent Resident Card, and as a sought-after immigration benefit, the U.S. Permanent Resident Card has been subject to countless attempts at unlawful replications and alterations over the years. With readily available access to digital printing methods such as thermal, inkjet, and laser printers, document mills have proliferated around the world. As a result, the United States Government has constantly worked to reinforce the security of the Card to frustrate and deter those who would seek to gain the benefits of the Card through unlawful means. Nevertheless, each year, the Homeland Security Investigations Forensic Laboratory (HSI-FL) examines a multitude of counterfeit Permanent Resident Cards.

The HSI-FL has a library that is home to thousands of genuine standard travel and identity documents including, but not limited to, passports, birth certificates, social security cards, and permanent resident cards. Because of the vast number of counterfeit Permanent Resident Cards received at the HSI-FL each year, United States Citizenship and Immigration Service (USCIS), the issuer of the Permanent Resident Card, has continually solicited HSI-FL support by means of counterfeit deterrence evaluations and adversarial analysis in order to design a more secure document.

Older generations of the Permanent Resident Card were unsophisticated, making them more vulnerable to counterfeiting or

alteration. The older cards simply contained a paper substrate with an attached photograph held within a laminated pouch. Newer versions of the card have incorporated an integrated photograph, holographic laminate, and an ultraviolet feature. As of May 2010, the Permanent Resident Card consists of a polycarbonate layer that is receptive to laser engraving. After the polycarbonate substrate has been fused by heat and pressure and the personalized data has been laser engraved, convincing alterations become difficult. The laser engraving becomes intertwined with the polymer of the card, making its removal unlikely, whereas in the older generations of the card, the paper substrate could more readily be separated and altered. Further, this newest version of the Permanent Resident Card contains a complex combination of Level 1 and Level 2 security features. (Note: A Level 1 security feature is one that can be observed without the use of instrumentation, whereas a Level 2 security feature requires the use of a simple tool). The Level 1 security features found within the Permanent Resident Card include an optically variable device, or hologram, optically variable ink, personalized data with tactility, and an optical memory stripe on the reverse side of the card. The Level 2 security features found within the Card include high resolution microprinting and an ultraviolet feature.

Document, Security Features, Counterfeit

J28 On the Forensic Value of Non-Original Signatures on Travel and Identity Documents

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After attending this presentation, attendees will have a better appreciation of the widespread use of non-original signatures as a security feature, including its limitations.

This presentation will impact the forensic science community by alerting policy makers and designers of secure travel and identity documents by highlighting the limitations of a common security feature.

The universal function of a signature is to provide evidence of: (1) the provenance of a document; or, (2) consent. Forensic document examiners are routinely called upon to analyze signatures for the purpose of determining authorship. In recent years, there has been a trend in the production of travel and identity documents toward inclusion of an image of the document-holder's signature; typically captured from a digital scan of an original ink-on-paper signature from the application document. Although promoted as a security feature, this image is more often than not a liability rather than an added feature of protection or personalization on the card.

There are many elements of a signature that are evaluated in the course of a forensic examination. Features such as the relative size, slope, and the ratio of glyph size can be assessed from an image or copy of a document, provided that the copy or image quality is not overly degraded. There are far more features that require viewing with the aid of magnification in order to properly assess. These features include line quality, tremor or hesitation, direction and sequence of stroke, retouching, pen stops or lifts, and relative speed. Even the evaluation of glyph design often requires some magnification.

National identity cards, passports, drivers' licenses, and health cards all are examples of identity documents that include some level of personalization. Typically, each of these documents includes a visible, printed image of a signature, and in recent years this image has been reproduced with very poor quality. The limitations on quality are not with technology since micro-printing on many different media and using various inking techniques is commonplace. The limitation is with popular understanding of the use and abuse of the signature.

As the evolution of travel/identity documents progressed from original ink on substrate signatures to digital reproductions, the actual affect of having a signature printed on an identity document has become a liability. These documents cannot be used to forensically compare signatures with legitimate, known sample signatures for the simple reason that so many elements critical to the evaluation of the signature are not present. They also may not be able to be used as legitimate, known samples of an individual's signature for that same reason.

Furthermore some travel/identity document issuing processes allow for alterations to genuine signatures. When images are resized to fit into a predetermined window or space (e.g., under a photograph) they are often compressed along either their *x* or *y* axis without respect to the original aspect ratio. Some signatures are cropped so that portions are deleted or removed in order to fit into this artificially constrained space. The resultant signature image does not even display those superficial features of a signature such as size and ratio that are relied upon by primary validators at ports of entry, for example. The signature portion of many travel/identity documents is unable to meet the requirement for validation to which similar on-board data such as biographical information and photographs are routinely subjected.

The goal of this presentation is to highlight the issues and limitations surrounding the forensic examination of these signature images from travel/identity documents.

Travel Document, Identity Document, Signature

J29 Alternative Methods for Dry Seal Analysis

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After attending this presentation, attendees will gain a better understanding of traditional and unconventional non-destructive methods to examine dry seal impressions.

This presentation will impact the forensic science community by demonstrating new and different types of methods that can be used when traditional methods of dry seal examination are unsuccessful or insufficient. The goal of this research is to determine under what circumstance each technique will demonstrate an advantage over the other.

A dry seal is a non-inked mechanical device which embosses a design on a substrate. A dry seal impression is fixed on the substrate by application of two complementary metal plates, one a positive die with the design of the seal raised on its surface, the other a negative die with the depressed design.[1] Historically, dry seal devices have been used by notaries to verify legal documents, as stationers' embossments to identify the paper manufacturer or business, on architectural or engineering construction documents, or land survey drawings, to certify the identity of the licensed professional who supervised the development.[2,3,4] Dry seals continue to play a role in the forensic science community and are used by forensic document examiners to assist in authenticating passports and other identification documents. They can be used as a security feature, associating a photograph, text, or signature to a page, by compressing or permanently affecting the paper fibers, making alteration or replacement difficult. Some forgers, rather than attempting to replicate the design of a genuine seal, use a similar seal to make a weak impression in hopes that no one will detect the deception.[1,2] The Homeland Security Investigations Forensic Laboratory (HSI-FL) is tasked with determining if a document has been altered, which may include authenticating dry seals on passports, birth certificates, and other types of identification documents.

The dry seal impressions used for this research will be obtained from a variety of sources, including laboratory casework, specimens from the forensic laboratory reference library, and self-generated specimens. This experiment will strive to incorporate impressions of varying degrees of quality and depth. The dry seal impressions will be placed into three categories based on the clarity of the impression. Impressions that have all of the details including the texts and pictographic elements distinctly visible will be placed into Category I, impressions that have some of the details visible will be placed into Category II, and Category III will include impressions with no observable details. The categorization will be reviewed by other examiners not associated with this research project. A diverse selection of methods and instrumentation, including the traditional methods using oblique lighting, the Video Spectral Comparator 6000 (VSC), microscopy and unconventional techniques using the Electrostatic Detection Apparatus (ESDA), and Reflectance Transformation Imaging (RTI) will be applied in this research. The toner bead cascade method, the toner pad, the

aerosol development hood, and a dry erase marker will be used in conjunction with the ESDA equipment. Reflectance Transformation Imaging (RTI) is a new imaging technique based upon the combination of multiple digital images of an object illuminated from different angles. The object and the camera are in a fixed position which allows for the creation of a composite image from several images. The results obtained from this research will expose forensic document examiners to other techniques available for dry seal examination.

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Dry Seals, ESDA, VSC, RTI, Microscope

J30 Application of Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) to the Analysis of Red and Green Permanent Marker Inks

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After attending this presentation, attendees will understand the forensic potential of Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) as a method of analysis for permanent marker inks. Attendees will also understand how Principle Component Analysis (PCA), Discriminant Analysis (DA), and Hierarchical Cluster Analysis (HCA) can be used to demonstrate the discriminatory capabilities of ATR-FTIR.

This presentation will impact the forensic science community by increasing awareness of the capabilities of ATR-FTIR for the analysis of inks.

The analysis of inks serves many important functions in forensic science, including the determination of document authenticity and the comparison of document sources. The tests often require identification of the manufacturer and the specific formula of the ink. A marker is classified as a permanent marker if its ink adheres to most surfaces, has water-resistant properties, and uses dyes or pigments. Permanent markers typically contain three main components: colorants, carriers, and resins.

Numerous analytical methods have been used in the analysis of inks, and particularly of their dyes and pigments. These methods include Thin-Layer Chromatography (TLC), Liquid Chromatography (LC), Capillary Zone Electrophoresis (CZE), Infrared spectroscopy (IR), Raman spectroscopy, surface-enhanced Raman spectroscopy, Mass Spectrometry (MS), and Gas Chromatography–Mass Spectrometry (GC/MS). Various combinations of these analytical techniques may also be used.

In this study, ATR-FTIR and TLC were used for the analysis of the inks from both red and green permanent markers from eight different brands. ATR-FTIR is rapidly becoming the preferred method for acquiring infrared spectra, because little sample preparation is required: samples may simply be placed on the ATR crystal and an infrared spectrum scanned. In this instance, permanent marker inks were applied to the dull side of a sheet of aluminum foil. After the inks had dried, the inked spots on the foil were pressed against the ATR crystal. The FTIR spectra were analyzed using principal component analysis (PCA), linear discriminant analysis (DA), and agglomerative hierarchical cluster analysis (HCA). PCA is a multivariate technique that analyzes a data set in which observations are described by many inter-correlated quantitative dependent variables. Its goal is create a new, smaller set of variables that represent the meaningful variation in the original data set.

After scaling and baseline corrections, the spectra of each brand of permanent marker ink showed remarkably high consistency. Each brand had a unique infrared spectrum. For PCA, the green permanent marker inks required five components to account for more than 90% of the cumulative variance. The red permanent marker inks required six components to account for more that 90% of the variance. In DA, both red and green attained 100% correct classification for the original groupings and 100% correct classification in cross-validated groupings. TLC was performed by making “scribble” sheets on filter paper. Seven millimeter punches were taken from each “scribble” sheet. One-half of each punch was extracted into 40 µL of methanol and the extracts were spotted on silica gel TLC plates. The TLC plates were developed with a mobile phase consisting of ethyl acetate:ethanol:water (75:35:30). The developed TLC plates were examined under both normal room light and under long wavelength ultraviolet light. For each color of permanent marker, each brand of marker produced a unique chromatogram. The discriminating power of TLC and ATR-FTIR were found in this instance to be identical.

This study demonstrates that high-quality ATR-FTIR spectra of permanent marker inks can be easily obtained and that these spectra readily discriminate different brands. Further research needs to be done on alternative sampling methods, on obtaining ATR-FTIR spectra *in situ,* and on the effects of the environment on the infrared spectra of the inks.

Infrared Spectra, Ink, Chemometrics

J31 An Experimental Study on Methods to Reveal the Pen Pressure Dynamic of Chinese Signatures

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WITHDRAWN

J32 ESDA Visualization of Marks Imparted by Postal Service Processing

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After attending this presentation, attendees will understand what types of indentations can be left by postal processing and how they may or may not differ from other indentations.

This presentation will impact the forensic science community by increasing understanding of all of the indentations found on document evidence, and being more informed as to the value of such markings.

Electrostatic Detection Apparatus (ESDA) examinations commonly reveal bands and/or lines on the documents being examined. Work done previously has established that the relative placement of some of those features corresponds to components in digital printing or photocopying processes (specifically, roller/feed mechanisms). A recent case at the Homeland Security Investigations–Forensic Laboratory (HSI-FL) involved numerous letters and envelopes, which were processed for indented handwriting using Foster & Freeman’s ESDA-2. Only one of the documents actually had indented handwriting, and that writing appeared to have been made by administrative staff who had received the item. In addition to the indented writing, a variety of bands and lines were revealed in the ESDAs, the significance of which was initially unknown to the examiners involved. A review of professional

meeting presentations and publications revealed the prior work about printer-generated marks.[1,2] Many of the marks in the case documents were consistent with the existing assessments of their likely sources. However, many of the items contain qualitatively different marks such as a wide band in the center of each half of the opened and flattened envelopes, and a wide band in the center of each third of the folded letters. It seems likely that those bands are artifacts of processing equipment used by the US Postal Service, since they had to have been created when the letters were inside the envelopes, and there are likely no other automated processes between sealing an envelope and its being sorted by the Postal Service's automated equipment in this particular case. Knowing that something is "likely" is not a sufficient understanding of the origin of these indentations and an analysis of the components of the processing equipment with an eye toward establishing what produces the wide band is required. Additionally, this type of analysis could reveal whether other Postal Service components could be producing other artifacts that would be revealed by an ESDA examination.

The experimental protocol includes preparing a number of test samples to include blank sheets of paper pulled from a common ream of paper, folding the sheets twice, and inserting them into addressed envelopes. A group of the samples are mailed from different locations using the United States Postal Service. Some samples from this group are mailed locally and some are mailed from the western United States, all of which arrive at the same location. Another group of samples is taken to the local Postal Service sorting facilities (including the regional Dulles sorting facility), and with the facility's permission, inserted into the sorting equipment to observe which specific mechanisms physically touch the samples in order to better understand what impressions are made. Finally, a third group of samples is used as a control. These samples are not exposed to any sorting equipment and are not put through the Postal Service at all. ESDAs are developed for all samples in each group and assessments are made as certain indentations are revealed.

This study is not meant to be exhaustive as it is not feasible to send mail through all Postal Service facilities. There are also countless combinations of equipment that a piece of mail can encounter, depending on the sending and receiving locations.

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Questioned Documents, Indentations, Postal Service

K1 Effects of Hair Bleachers in the Analysis of Amphetamine(s) and Bath Salt Drugs

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After attending this presentation, attendees will learn about the effects of hair bleaching in the analysis of amphetamines and synthetic cathinones in hair using readily available Solid Phase Extraction (SPE) cartridges and tandem mass spectrometry.

This presentation will impact the forensic science community by offering analysts operating in forensic facilities information about the impact of bleaching materials used on hair analyzed for amphetamines and synthetic cathinones (bath salt) drugs analyzed by Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) and solid phase extraction.

Method: Samples of decontaminated hair (10mg) containing amphetamines and bath salt-type drugs (amphetamine, methamaphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), butylone, ethylone, flephedrone, mephedrone, methylone, methedrone, methcathinone (4-MEC), methylenedioxypyravalerone (MDPV), and pyravalerone were treated with 10% aqueous sodium hypochlorite solution (bleach), 3% aqueous hydrogen peroxide solution, or 3% aqueous ammonium hydroxide solution for 2 hr before being removed, washed, and dried. The samples were then digested in 0.1M NaOH (containing deuterated analogues) for 0.5 hr at room temperature. Each solution was adjusted to pH6 with 0.1M phosphate buffer (4mL) and applied to a conditioned SPE column. The samples were extracted on commercially available SPE columns (C8/SCX). After loading the sample, the sorbent was washed with deionized water, acetic acid (0.1M), and methanol (3mL of each, respectively). Each SPE column was dried and eluted with 3mL of a solvent consisting of methylene chloride/isopropanol/ammonium hydroxide (78:20:2). After elution, 200µL of mobile phase was added to the collection tube. The samples were then evaporated to the mobile phase for analysis by LC/MS/MS in positive Multiple Reaction Monitoring mode (MRM). Data is presented for MRMs of amphetamine, methamphetamine, MDA, and MDMA, butylone, ethylone, flephedrone, mephedrone, methylone, methedrone, methcathinone (4-MEC), methylenedioxypyravalerone (MDPV), and pyravalerone (and deuterated analogues), respectively.

Liquid chromatography was performed in gradient mode employing a 50 x 2.1mm C_{18} analytical column and a mobile phase consisting of acetonitrile and 0.1% aqueous formic acid. The gradient was programmed to run from 5% to 90% acetonitrile in 4.0 min and then back to 5% for re-injection. The total run time for each analysis was less than five minutes.

Tandem mass spectrometry was performed in using positive MRM mode. The following transitions were monitored (quantification ions underlined): Amphetamine m/z: 136.1 to 91.0, 65.0, Amphetamine-d_5: m/z 141.1 to 124.0, 93.0, Metamphetamine: m/z 150.1 to 91.1, 119.1 Methamphetamine-d_5: m/z 155.2 to 92.1, 121.2, MDA: m/z 180.2 to 163.1, 105.1, MDA-d_5: m/z 185.2 to 168.1, 110.1, MDMA: m/z 194.2 to 163.1. 105.1. MDMA-d_5: m/z 199.2 to 165.1, 106.8, Butylone: m/z 222.1 to 174.2, 204.2, Ethylone: m/z 222.1 to 174.2, 204.2, Flephedrone: m/z 182.1 to 164.2, 149.1, Mephedrone: m/z 178.1 to 145.1, 160.1, Methylone: m/z 208.1 to 160.1, 132.1, Methedrone: m/z 194.1 to 176.2, 161.1, Methylethylcathinone (4-MEC): m/z 192.1 to 174.2, 144.1, Methylenedioxypyravalerone (MDPV): m/z 276.2 to 135.1, 126.1 and Pyravalerone: m/z 246.2 to 105.1, 175.2, respectively. In this presentation, representative chromatograms are shown to illustrate the efficiency of the chromatography and analysis of amphetamine and synthetic cathinones

Results: The limits of detection/quantification for the SPE method were determined to be 0.05ng/mg and 0.1ng/mg, respectively for the amphetamines (amphetamine, methamphetamine, MDA, and MDMA) and synthetic cathininones (butylone, ethylone, flephedrone, mephedrone, methylone, methedrone, methcathinone (4-MEC), methylenedioxypyravalerone (MDPV), and pyravalerone). The method was found to be linear from 0.1ng/mg to 10ng/mg (r^2>0.999). Data is presented to show that recoveries of amphetamine were found to be greater than 90%. Interday and Intraday analysis of amphetamine were found to <7% and <10%, respectively. Matrix effects were determined to be <5%. Degradation of the amphetamines ranged from 77% to 45%, while the degradation of the synthetic cathinones ranged from 25% to 100% for the bleaching agents.

Conclusion: The use of the information given in this new procedure for the analysis of amphetamine and synthetic cathinones will be of great use to analysts in the field of forensic hair analysis as it demonstrates the use of SPE/LC/MS/MS to provide valuable data from about the effects of bleaching agents in hair analysis.

Hair, LC/MS/MS, SPE

K2 Extraction and Analysis of AM2201 Metabolites in Urine: A Drugs and Driving Case

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After attending this presentation, attendees will learn about the extraction and analysis of the hydroxy metabolites of AM2201 (a newer fluorinated synthetic cannabinoid) from urine using readily available Solid Phase Extraction (SPE) cartridges and tandem mass spectrometry.

This presentation will impact the forensic science community by offering forensic toxicology analysts and chemists involved with drugs and driving cases more information about the analysis of this synthetic cannabinoid (AM2201 hydroxylated metabolites) employing LC/MS/MS and solid phase extraction.

Method: In this method, urine (1mL samples (calibrators, controls, and test samples)) containing internal standard (THC-d_3/AM2201-OH-d_5) was enzymatically hydrolysed with β-glucuronidase before being adjusted to pH6 with 0.1M phosphate buffer. The samples were then applied to pre-conditioned SPE mixed mode columns (C_8-Weak Anion Exchange). The SPE columns were conditioned with methanol, dionized (DI) water, and 0.1M pH6 phosphate buffer (3mL, 3mL, 1mL, respectively). After loading the samples onto the columns, the SPE sorbents were washed with DI water and pH6 phosphate buffer containing 20% acetonitrile (v/v) (3mL of each) and then SPE columns were dried under full vacuum for 5 min. The analytes were eluted with 3mL of a solvent mixture consisting of ethyl acetate containing 10% methanol. The eluates were evaporated to dryness using nitrogen gas at 40°C and dissolved in 100µL of a mixture of: 95% aqueous formic acid (0.1%) and 5% acetonitrile (containing 0.1% formic acid). The samples were

analyzed by tandem mass spectrometry using positive Multiple Reaction Monitoring mode (MRM) and gradient liquid chromatography. Liquid chromatography was performed on a 50x2.0mm C_{18} analytical column with a guard column of the same chemistry. The mobile phase employed consisted of **A** aqueous formic acid (0.1%) and **B** acetonitrile (containing 0.1% formic acid). The gradient was started at 5% **B** and increased 90% **B** in 4 min, after which it was decreased to 5% **B** and kept until 5 min. The flowrate of the mobile phase was 0.5mL per min. Each analytical run was completed in 5 min.

In this presentation, representative chromatograms and calibration curves are shown to illustrate the efficiency of the chromatography and analysis of AM2201 and its metabolites.

Results: The limits of detection/quantification for the SPE method were determined to be 0.5ng/mL and 1.0ng/mL, respectively for the analytes The method was found to be linear from 1.0ng/ mL to 100ng/ mL (r^2>0.999). Data is presented to show that the recoveries of the AM2201 metabolite were found to be greater than 90%. Interday and intraday analysis were found to <7% and <10%, respectively. Matrix effects were determined to be <5%. No parent drug was found in the test sample. Results of the metabolite concentrations are shown in the presentation.

Conclusion: The use of the information given in this new procedure for the analysis of the metabolites AM2201 will be of great use to analysts in the field of forensic toxicology as it demonstrates the use of SPE/LC/MS/MS to provide valuable data regarding the metabolites of one of the newer synthetic cannabinoids

AM2201, SPE, LC/MS/MS

K3 Methylenedioxypyrovalerone (MDPV) Postmortem Blood Concentrations: A Series of Suicide Case Reports

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The goal of this presentation is to gain an understanding of the analytical methods employed to qualitatively identify and quantitatively analyze postmortem specimens for the presence of MDPV and other related “bath salt” compounds. Additionally, attendees will gain insight into the interpretive relationships of MDPV postmortem blood concentrations in deaths where drugs were not a factor.

This presentation will impact the forensic science community by emphasizing these unique case histories and circumstances, frequently characterized with atypical or bizarre decedent behaviors, which can direct postmortem toxicological testing strategies to the analyses of MDPV and other “bath salt” compounds.

Methylenedioxypyrovalerone (MDPV), a synthetic beta-ketone resembling methylmethcathinone (mephedrone) and its analogs (commonly termed “bath salts”), has a worldwide distribution and is promoted as a “legal high.” Dubbed “not for human consumption,” these stimulant agents are easily purchased from a number of sources to include convenience stores, “head shops,” and the internet. The agents are identified as “bath salts,” “plant food,” “jewelry cleaner,” and “pipe cleaner” in an apparent attempt to circumvent laws regulating psychostimulant substances. MDPV is a stimulant chemically related to methylphenidate and methylenedioxymethamphetamine (MDMA); its pharmacological mechanism of action is similar to cocaine in acting as a dopamine and norepinephrine re-uptake inhibitor. The products are marketed with clever trade names (e.g., “Ivory Wave,” “Vanilla Sky,” “Hurricane Charlie,” and “Bolivian Bath”) and colorful creative packaging. Emergency departments and poison control centers throughout the United States have reported epidemic-like encounters characterized as sympathomimetic toxidromes accompanied by profound mental status and behavioral changes in users. Clinical case reports have also described paranoid psychosis and hallucinatory delirium following the use of MDPV. Persons intoxicated or under the influence of MDPV or other “bath salts” pose keen interest in forensic postmortem and human performance cases; however, routine toxicology screening techniques may not be adequate for detection and identification of these compounds. This study reports an analytical technique for the qualitative and quantitative analyses of MDPV in postmortem specimens. The method is suitable for the analyses of other “bath salt” compounds and is performed routinely by some laboratories in postmortem and human performance cases. The toxicological findings are presented for five postmortem cases submitted to a laboratory between February and November 2011, which include the quantitative analyses of MDPV in blood. The manner of death in all cases was suicide. Three of the cause-of-death classifications involved self-inflicted gunshot wounds, while two cases were attributed to hanging. Three of the five cases were male, with an age range of 25 – 36 years for the five decedents. The range observed for the concentration of MDPV in postmortem blood is 68.3 to 1,044ng/mL. MDPV and other “bath salt” compounds were extracted via a protein precipitation extraction with acetonitrile. Instrumental analysis utilized in identifying and quantifying MDPV was Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS). Three of five cases exhibited other positive toxicological findings (ethanol, benzodiazepines, THC-COOH, opiates and opioids); one case included detection of mephedrone in blood and other postmortem specimens.

Apparent recreational use of MDPV and other “bath salt” derivatives and analogs is an emerging substance abuse problem. The index of suspicion should be high among forensic pathologists, medical examiners/coroners, and toxicologists when a case history is characterized by bizarre, delusional, and hallucinatory decedent behavior. Profound, intentional self-harm with fatal consequence is described for the five cases in this series of reports; MDPV was quantitatively reported for postmortem blood in all cases, and witness accounts for two of the cases confirmed decedent use of “bath salts” prior to death.

MDPV, Blood, Suicide

K4 Fatal Overdose With the Anti-Diarrheal Medication Loperamide

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After attending this presentation, attendees will be briefed on an apparent suicidal overdose involving loperamide, including analytical parameters used for confirmation and quantitation.

This presentation will impact the forensic science community by describing lethal loperamide concentrations and autopsy observations. This case also highlights the importance of scene investigation and communication between the toxicology laboratory and medical examiners.

Loperamide is a Piperidine Opioid (PO) found in several over-the-counter anti-diarrheal preparations. Doses range from 2 – 4mg PO in adults, not to exceed 16mg daily and expected plasma concentrations are <0.01mg/L. At therapeutic doses, loperamide does not produce typical opioid effects on the central nervous system because of low systemic availability, high protein binding, and poor accumulation in the brain. Loperamide and its primary, inactive metabolite desmethylloperamide are almost immediately pumped out of the brain by P-glycoproteins. Adverse effects are rare, but can include cramps, nausea, drowsiness, dizziness, headache, and dry mouth. Overdoses are usually accidental, nonfatal, and occur in children under age three. Here is reported an apparent suicidal overdose involving loperamide.

A 20-year-old White male with a history of pain and depression was found dead at home along with eight empty 72-count bottles of 2mg loperamide hydrochloride tablets and a receipt of purchase dated

one day prior. No suicide note was found. Significant autopsy findings included clear, frothy fluid at the lips and slight vomitus on the face. Biological tissues and fluids collected at autopsy were submitted for toxicological analysis with loperamide noted as a suspected factor in death.

Femoral blood and urine were negative for ethanol, methanol, acetone, and isopropanol. Enzyme-linked immunosorbent assay screening for cocaine metabolite, opiates, methamphetamine/MDMA, phencyclidine, barbiturates, carisoprodol/meprobamate, fentanyl, methadone, zolpidem, amphetamine/phentermine, acetaminophen, and salicylate was negative in femoral blood. HPLC-UV analysis tentatively identified 7-aminoclonazepam, but was not confirmed by mass spectrometry. Alkali-extractable drug screening by liquid-liquid extraction and GC/MS was also negative.

Given the numerous empty bottles on scene and autopsy findings suggesting overdose, it was assumed that loperamide may be present in this case but instrument settings precluded identification as loperamide and desmethylloperamide are reportedly late eluters. As hypothesized, loperamide fortified into drug-free blood, extracted and analyzed by a normal GC/MS screen was not detected. By extending the final hold time, loperamide eluted with substantial tailing. Modifying the temperature ramp further improved peak shape and loperamide was confirmed in femoral blood and urine. Desmethylloperamide was tentatively identified by spectral library match in both matrices, but a reference standard is not commercially available for comparison.

This study successfully quantitated loperamide by GC/NPD using a less polar column and the optimized GC/MS chromatographic conditions. The linear dynamic range was from 0.1 – 6mg/L and accuracy was greater than 85%. Loperamide results obtained in blood, urine, liver, and gastric contents were 0.4mg/L, present, 7mg/kg, and 30mg/kg, respectively; urine was evaluated qualitatively per department policy.

The medical examiner ruled the death a suicidal overdose of loperamide. Two suicidal loperamide overdoses are reported with loperamide concentrations of 2.6mg/L (blood), 12.5mg/kg (liver), and 3mg/kg (gastric contents) in one case and 0.084mg/kg (blood) and 0.87mg/kg (liver) in the other. Blood concentrations in this case and the literature greatly exceed expected therapeutic concentrations. Presumably in overdose, the P-glycoprotein efflux mechanism is overwhelmed, allowing loperamide to exert typical opioid CNS effects leading to euphoria and ultimately death.

Internet-user forums debate the effectiveness of high-dose loperamide to achieve euphoria, ward off withdrawals, and potentiate other opioid receptor agonists. If euphoria is achieved, the high is reportedly not as intense as other prescription opiates/opioids and does not justify the cost or gastrointestinal side effects. Still, since loperamide is easily accessible, the potential for abuse exists and laboratories should evaluate whether their basic drug screens are capable of detecting this late-eluting compound.

Loperamide, Suicide, Death Investigation

K5 Analysis of a Group of Volatile Compounds With Forensic Interest: Validation of an Analytical Method by HS-GC/FID

Carla Monteiro, BS, Miguel Franco, MSc, Cristina Cordeiro, MSc, Alda Claro, BS, Paula Proença, BS, Carla Mustra, MS; and Francisco Corte-Real, PhD, Instituto Nacional de Medicina Legal, Largo da Sé Nova, Coimbra, 3000-213, PORTUGAL; and Duarte N. Vieira, PhD, MD, Rua Antonio Jose de Almeida, No 117, Coimbra, 3000-044, PORTUGAL*

After attending this presentation, attendees will understand the potential contribution of a new method for detection and quantification of volatile substances in different biological matrices with interest in forensic contexts.

This presentation will impact the forensic science community by allowing toxicology experts to understand the specificities and difficulties of validating an analytical method developed for the analysis of volatile compounds with different solubilities like toluene or acetone.

Although pharmaceutical products, drugs of abuse and ethanol (alcohol) are the most common poisons encountered in clinical and forensic toxicology, the possibility of poisoning with a wide range of other compounds has to be taken into account. These include pesticides, volatile substances, metals and anions, and natural toxins.

The purpose of this work was the optimization and validation of a sensitive and rapid analytical procedure to the detection and quantification of some volatile organic compounds (acetaldehyde, ethyl acetate, acetone, acetonitrile, 1-butanol, diethyl ether, methanol, 2-propanol, chloroform, toluene and xylene) in different matrices (blood, urine and vitreous humor) using a gas chromatograph, equipped with a flame ionization detector coupled to a headspace injector of fixed volume (1mL loop) Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID).

The substances under study were divided and grouped according to their solubility and working range. For substances with high water solubility, a mixture was created (acetaldehyde, ethyl acetate, acetone, acetonitrile, 1-butanol, diethyl ether, methanol, 2-propanol). The other substances, whose solubility in water was practically non-existent but had a good solubility in methanol, were divided according to the working range.

Prior to gas chromatographic analysis, all specimens, including the calibrators, were diluted 1:10. By volume, i.e., 100µL of urine, vitreous humor, or blood were diluted with 1mL aqueous solution of n-propanol (100mg/L), used as internal standard.

The chromatographic separation was performed using two capillary columns with different polarities, in order to ensure fulfillment of the identification criteria recommended for this type of analysis (Flanagan *et al*., 1997; Kugelberg *et al*., 2007).[1,2] Chromatographic analysis conditions were as follows: an initial oven temperature of 40°C, held for 5 min, followed by a rise to 130°C with a gradient of 10°C/min. At the end of each analytical cycle, the initial conditions were resumed and maintained for 3 min. The injector temperature was maintained at 150°C, with a split ratio of 4:1, with detectors set at 250°C. The carrier gas was helium at a constant flow rate of 2.7mL/min.

All compounds studied, including n-propanol (internal standard), eluted in a time interval of 15 min and were all well resolved with no interference of metabolites, degradation products, or other substances, such as t-butanol, formaldehyde, ethylene glycol, methyl and ethyl formate, etc. In the concentration ranges analyzed, and for all compounds, the analytical response proved to be linear with a correlation coefficient greater than 0.9962. The limits of detection varied between 1mg/L (1-butanol, toluene, and xylene) and 10mg/L (chloroform) and the limits of quantification between 2mg/L (xylene) and 31mg/L (chloroform). The coefficients of variation obtained for intermediate precision varied from 0.8% (acetonitrile) to 7.0% (xylene). The accuracy of the method varied between 87.8% (acetaldehyde) to 106.3% (xylene).

The study focused on all parameters included in the validation procedure for quantitative methods, in place at the forensic toxicology laboratory of Centre Branch—Portuguese National Institute of Legal Medicine. These included the study of selectivity, linearity, limits of detection and quantification, precision, accuracy, robustness and carryover, having the method shown to be suitable for the intended purpose.

References:

1. Flanagan, R.J.; Streete, P.J.; Ramsey, J.D. (1997), Volatile substance abuse; practical guidelines for analytical investigation of suspected cases and interpretation of results, UNDCP *Technical Series* No 5, United Nations Drug Control Programme, Vienna.

2. Kugelberg, F.C.; Jones, A.W. (2007), Interpreting results of ethanol analysis in postmortem specimens: a review of the literature, Forensic Science International 165, 10-29.

HS-GC/FID, Volatile, Validation

K6 Applications of Hydrophilic-Interaction Chromatography in Forensic Science

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After attending this presentation, attendees will have a better understanding about hydrophilic interaction liquid chromatography and review of literature displaying how this method can be used for the forensic science community.

This presentation will impact the forensic science community by providing information on the application of Hydrophilic Interaction Liquid Chromatography (HILIC) to the separation of analytes in different matrices including forensic drug and toxicological samples.

HILIC is a mixed or multi-modal partition chromatography designed specifically to separate polar, ionic, or weakly acidic and basic compounds. The aqueous/organic mobile phase is passed over the more polar stationary phase. Columns consist mainly of bare silica or chemically bonded silica such as simple amide, cyano, and diol to complex alkyl and polymeric coatings. The columns can be particle packed or monolithic. The bonded moieties can range in thickness to allow for specific aqueous saturation. The aqueous layer creates electrostatic repulsion and other intermolecular forces to aid in the separation process of more similar compounds such as isomers.

The highly organic mobile phase is composed mainly of acetonitrile and can be controlled through gradient or simple isocratic elution. High concentrations of organic modifiers allow for proper ionization of analytes and, therefore, are compatible with an electrospray ion source of a mass spectrometer. Due to advances in mass spectrometry, this is the detector type of choice when looking at low concentration of analytes and analytes in difficult matrices such as whole blood. As an alternative to normal and reverse phase liquid chromatography, HILIC sustains selectivity and prominent peak shape while using rapid isocratic methods.

Reversed reverse-phase or aqueous normal phase chromatography was coined HILIC by A.J. Alpert in 1990. The first applications of HILIC were primarily of bio analytics such as proteomics and metabolomics because of the ability to purify bio markers, amino acids, and other proteins. In the pharmaceutical industry, the use of HILIC has grown for purposes such as quality control and processes pertaining to research and development. Although HILIC is not a new technique, this form of chromatography is beginning to become more prevalent because of stationary phase developments. Advances in stationary phase preparation, including nanostructures within polymeric scaffold, create efficient preparation and productive permeability. The new production techniques allow not only for a variety of moieties, but also lower cost and create a more consistent product. The separation efficiencies and increased production of HILIC columns has significantly amplified research and applications. Today, HILIC can be seen to be applied to broader applications. HILIC has been applied to many fields, including forensic science and forensic toxicology. Designed for polar metabolites, HILIC is a valuable asset to forensic toxicologists for the analysis of such polar drug metabolites.

Reviews of forensic HILIC applications are seen in such studies as comparison of ethyl-glucuronide distribution in pubic and head hair. Other topics include body fluid and tissue distribution of cocaine and associated metabolites. Estimations of *g*-hydroxybutyrate levels in serum also use HILIC. A method for screening and confirming stimulants, narcotics, and beta-adrenergic agents in urine used the capabilities of HILIC. A comprehensive review for HILIC of seized drugs and related compounds by the Drug Enforcement Agency is also cited. The advantages of HILIC separation of isomers such as morphine-6-glucurinde and morphine-3-glucuronide are also presented. This presentation will review general theory and forensic applications of HILIC for the past 10 years.

HILIC, Forensic, HPLC

K7 Analysis and Characterization of the First- and Second-Generation Raving Dragon Novelty Bath Salts

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After attending this presentation, attendees will see an example of how quickly a single brand of bath salts can change its ingredients as well as the spectroscopic characterization of 3,4-methylenedioxy-N-methylcathinone (methylone) and 2-methylamino-1-phenylpentan-1-one (pentedrone).

This presentation will impact the forensic science community by illustrating an example of the change in active ingredients found in bath salt preparations after the administrator of the Drug Enforcement Administration (DEA) issued a temporary schedule for three synthetic cathinones under the Controlled Substances Act (CSA). These substances were: 4-methyl-N-methylcathinone (mephedrone), 3,4-methylenedioxy-N-methylcathinone (methylone), and 3,4-methylenedioxypyrovalerone (MDPV). Also presented will be the Mass Spectrum, Nuclear Magnetic Resonance (NMR), Ultraviolet (UV), and Infrared (IR) spectroscopic characterization of the first-generation methylone and the second-generation 2-methylamino-1-phenylpentan-1-one (pentedrone), the lone ingredients found in the bath salts sold under the brand “Raving Dragon.”

Introduction: In recent years, a large number of new, uncontrolled designer drugs have appeared on the market. Several of the new synthetic drugs that are sold as bath salts belong to one of the classes of β-ketophenylethylamines or phenethylamines. These drugs are available in small packets containing milligrams to gram quantities. They are available via the internet or at various convenience stores, gas stations, truck shops, tattoo parlors, and discount tobacco outlets and are often sold as bath salts with the disclaimer, “Not For Human Consumption.”

In February of 2011, several packets containing 0.3g of an off-white powder sold under the name Raving Dragon Novelty Bath Salts were obtained via a website of the same name. This product was removed from the market in October of that year, coinciding with the DEA issuing a temporary schedule of mephedrone, methylone, and MDPV under the CSA. Four months later in February of 2012, a new bath salt was released from the same company under the new name Raving Dragon Voodoo Dust; again several packets were obtained containing 0.5g of a fine white powder.

Methods: Both products were subjected to various spectroscopic techniques: mass spectroscopy (Shimadzu MDGCMS QP-2010 Ultra), NMR spectroscopic (Bruker Ultrashield Plus-400MHz), UV (Shimadzu UV-1601 UV-Visible Spectrophotometer), and IR spectroscopy (Thermo Scientific Nicolet IS10), for the characterization and identification of the active ingredients in the packets. Once the spectroscopic techniques results were obtained for the active ingredients, these results were compared to reference standards in order to confirm their identity and purity.

Results: It was determined that the first-generation Raving Dragon Novelty Bath Salts contained methylone, one of the three compounds added to the banned substance list in October of 2011. The second-generation novelty bath salt, Raving Dragon Voodoo

Dust, was found to contain pentedrone. At the present time, pentedrone is unscheduled by the DEA. The purity of the bath salts was determined by UV using the specific absorbance (defined as the $A_{1cm}^{1\%}$ value) of the reference standard vs. the bath salt. Methylone with an $A_{1cm}^{1\%}$ of 550 at λ= 235 was determined to be 89% of the ingredients of the Raving Dragon Novelty Bath Salts. Pentedrone with an $A_{1cm}^{1\%}$ of 579 at λ= 256 was calculated to be 100% of the ingredients of the Raving Dragon Voodoo Dust.

Discussion: Recently, numerous articles relating to the pharmacological and toxicological effects of methylone, including several postmortem cases, have been published. Pentedrone has been previously identified in samples intercepted by the Canada Border Services Agency, customs in Berlin, and police organizations in several federal states of Germany. No specific pharmacological and toxicological data is available.

Conclusion: Once a synthetic compound or group of synthetic compounds are added to the DEA list of scheduled compounds, new analogs appear in their place. In the case of the Raving Dragon Novelty Bath Salts, methylone was replaced within four months of its scheduling with pentedrone as the active ingredient. Pentedrone should be added to the fast-growing group of "Legal High" (designer) drugs that can be expected to be found in bath salt products. The analysis of the Raving Dragon Brand Bath Salts illustrates the rapidly changing active ingredients in "Legal High" preparations that are readily available to the public.

This project was supported by the National Institute on Drug Abuse (NIDA) Center for Drug Abuse Grant P50DA005274.

Bath Salts, Methylone, Pentedrone

K8 Characteristics of Toxicology Laboratories Performing Drug-Impaired Driving Casework

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After attending this presentation, attendees will be able to describe the characteristics of U.S. laboratories performing toxicological analysis in drug-impaired driving cases, focusing on their size, workload, turnaround time, and level of engagement in training. The purpose of presenting this data is to allow attendees to evaluate the findings of a survey of toxicology laboratory users and compare how their own laboratory performs relative to others in terms of size, requests for testimony, and available instrumentation.

This presentation will impact the forensic science community by improving the understanding of expectations and needs of clients of Driving Under the Influence of Drugs (DUID) testing laboratories and standards of service delivery within the field.

Cases involving suspected DUID contain several important elements, including: an officer trained in documenting observations regarding the driving and subject behavior as well as the collection of a biological specimen for comprehensive toxicology testing; a toxicology lab to analyze the specimen for illicit substances; and a prosecutor to utilize the data in the court system. Three surveys were conducted, in collaboration with the National Safety Council, to poll State's Drug Recognition Experts (DREs), Traffic Safety Resource Prosecutors (TSRPs), and toxicology labs with the purpose of gathering information about the needs of the traffic-safety community regarding drug testing and testimony in DUID/DRE cases. TSRPs, DREs, and toxicology lab directors from each state at various jurisdictional levels were surveyed to identify areas of need in the scope and sensitivity of testing available, turnaround time, training, expertise for trial or preparation, meeting court-imposed deadlines, and other service factors of unmet need in training and research for scientists, law enforcement, and prosecutors.

In terms of staffing, the reported mean (median) size of the labs surveyed was 8 (6.5) analysts per lab (range 1-200), with a reported mean (median) of 74 (25) DUID/DRE cases each month per laboratory (range 1-1800). As expected, the size of the lab and the resources available affected the average. In terms of turnaround time, both DREs and TSRPs reported an average turnaround time of eight weeks with respect to drug analysis, and corresponding satisfaction ratings started to decrease among the DREs and TSRPs when turnaround time reached six to eight weeks. According to prosecutors, toxicologist's testimony affects trial outcome a reported average of 63% of the time, and there has been an increase in toxicologist appearances in court due to the confrontation clause issues which in turn contributes to an increase in the analytical backlog in the lab due to analysts being called to testify.

When asked about whether toxicologists are involved in DRE or TRSP training, only 53% of the respondents said they were involved. This type of training entails educating the DREs and prosecutors of what type of testing is provided, specific drugs that are tested for, understanding reports, statistics on drugged driving, and, for prosecutors in particular, how results in a given case are interpreted. When asked why a toxicologist isn't involved in DRE or prosecutor training, the majority reported that they haven't been asked, while others reported that it wasn't seen as necessary or there is insufficient staffing, funds, or resources. In addition to training among the DREs and prosecutors, toxicology labs also reported an additional need for training among the staff. The greatest areas of need for training include instrumentation, uncertainty determination, confirmation testing, and mock-trial training. Other high priorities for additional resources reported by the toxicology laboratories include additional staffing, instrumentation for confirmation, and upgrading or obtaining a new facility.

DUID, Lab Management, Testimony

K9 Sertraline in Postmortem Blood and Liver: Deaths in North Carolina (2002 – 2011)

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After attending this presentation, attendees will have greater insight into the different types of postmortem casework associated with sertraline and norsertraline at therapeutic and toxic concentrations.

This presentation will impact the forensic science community by providing information regarding sertraline and norsertraline concentrations as it relates to cause and manner of death determinations.

Sertraline (Zoloft®) is a Selective Serotonin Reuptake Inhibitor (SSRI) used in the treatment for depression in typical, daily adult doses ranging from 50 – 200mg. Patients on chronic oral daily doses of as much as 300mg reached a steady-state plasma level averaging 0.206mg/L (0.099-0.309mg/L). Sertraline undergoes N-demethylation to norsertraline which has about 10% – 20% the pharmacologic activity of its parent.

At the North Carolina Office of the Chief Medical Examiner, cases suspicious for toxicological cause or with essentially negative autopsy findings are routinely screened for common over-the-counter, prescription, and illegal drugs via various laboratory techniques. A search of the database for sertraline/norsertraline liver data with or without corresponding blood data revealed upwards of 200 cases over a 10-year period. Decedents were divided into three groups

according to the classification of the effect of sertraline as it impacts the cause of death. The pathologist considered sertraline to be either the primary cause of death (below), additive to the cause of death, or not implicated in death. The foci of the study are the overdose cases where sertraline was determined to be the primary cause of death regardless of other drugs and their concentrations (N=30) and non-overdose cases where sertraline received no classification (N=140). The latter focus may be considered postmortem normal concentrations.

Sertraline metabolism and elimination could be altered by the health of the patient, drug-drug interactions, and genetic deficiencies. The concentrations of sertraline and norsertraline, as well as the parent/metabolite ratio, were reviewed in light of pathological findings and co-intoxicants. Case studies involving significant postmortem redistribution and potential drug interactions will be highlighted.

Sertraline Overdose Cases (Suicide)

Specimen Location	N	Range		Average		Median	
		Sertraline	Norsertraline	Sertraline	Norsertraline	Sertraline	Norsertraline
Central (mg/L)		0.49-25	0.32-8.2	4.19	2.28	1.85	1.5
Peripheral (mg/L)		0.74-9.2	0.27-4.6	2.86	1.79	1.75	1.55
Liver (mg/kg)	20	27-490	5.4-940	137	127	94	72.5

Sertraline Overdose Cases (Accident)

Specimen Location	N	Range		Average		Median	
		Sertraline	Norsertraline	Sertraline	Norsertraline	Sertraline	Norsertraline
Central (mg/L)		0.61-3.1	0.93-4	1.16	1.92	0.82	1.6
Peripheral (mg/L)		0.74-1.8	0.36-3.7	1.21	1.71	1	1.3
Liver (mg/kg)	10	20-279	15-518	90.2	166.8	63.5	155

Sertraline, Death Investigation, Toxicology

K10 The Effects of Burn Injury on Tissue Ethanol and Ethyl Glucuronide Concentrations

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After attending this presentation, attendees will better understand the effects of burn injury on tissue ethanol and ethyl glucuronide concentrations after using a series of burn injury experiments to mimic a residential house fire and determine if visual appearance or core body temperature correlates to changes in analyte concentrations.

This presentation will impact the forensic science community by providing insight to the potential changes in ethanol and Ethyl Glucuronide (EtG) concentrations after burn injury and potential inaccuracies for determining impairment using post-incineration tissue ethanol concentrations.

Ethanol is a popular, legal drug and its deleterious cognitive effects cause an increased risk for residential house fires. Currently, there is no known data available to validate tissue ethanol and EtG concentrations and their interpretations in fire-related death victims. Tissues collected at autopsy must be used for toxicological analysis when blood is not available. The literature does not address the possibility that antemortem tissue ethanol or EtG concentrations maybe altered in fire deaths.

The main objective was to determine if exposure to a house fire causes changes in postmortem ethanol and EtG concentrations from antemortem concentrations.

Methodology included a Sprague Dawley rat model being used to determine the effect of burn injuries, using two fire-related models, on liver, kidney, and heart ethanol and EtG concentrations. The rodents were gavaged with ethanol (4g/kg) then euthanized after three hours by carbon dioxide. Burn injuries from fire deaths were mimicked using the reported average response time by local fire departments and two types of burn injury using a fire pit and a gas grill with these conditions:

Flame burn injury (Fire Pit) (n=9 per group, 3 groups) Temperature: >1000°F	Duration (minutes): 2, 5, and 8
Thermal burn injury (Gas Grill) (n=9 per group, 9 groups) Temperature: 200°F, 400°F, or 600°F	Duration (minutes): 2, 5, or 8

Homogenized specimens were analyzed for ethanol by Gas Chromatograph/Flame Ionization Detector (GC/FID) and EtG by enzyme immunoassay. Tissue ethanol and EtG concentrations from burn injury groups, non-ethanol dosed controls, and non-burn injured controls were compared to determine if any differences occurred in analyte concentrations due to flame and/or thermal burn injury. Core body temperatures were monitored using a rectal probe to determine if a correlation existed between changes in analyte concentrations and maximum core body temperatures.

The result was a significant time/temperature increase in tissue ethanol concentrations from both burn injury models. Only the greatest exposure to burn injury with both models produced a significant increase in EtG concentrations. Lesser time/temperature exposures produced a significant decrease in liver ethanol and kidney EtG concentrations. Tissues collected from non-dosed controls did not have detectable ethanol or EtG concentrations produced by burn injury. Changes in ethanol and EtG concentrations and organ weights did not correlate, but changes may be related to maximum core body temperature. Maximum core body temperatures ranged from 96°F – 151°F for burn injury groups.

In conclusion, the burn experiments using a rodent model suggest that caution should be used when predicting ethanol impairment from postmortem fire victim tissue ethanol and EtG concentrations because ethanol and EtG concentrations maybe altered from burn injury. In addition, it was determined that false positives are unlikely in individuals who have not consumed ethanol. This study was unable to determine the mechanism by which the changes in analyte concentrations were altered in corpses exposed to flame or thermal burn injury.

Burn Injury, Ethanol, Ethyl Glucuronide

K11 Blood Glucose Concentrations After Burn Injury

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After attending this presentation, attendees will have a better understanding of burn injury effects on fire victims' blood glucose concentrations.

This presentation will impact the forensic science community by providing an understanding of the potential changes that can occur in post-incineration blood glucose concentrations compared to antemortem blood glucose concentrations in fire-related deaths after burn injury.

Changes in postmortem biochemistry make interpreting toxicology results difficult when attempting to predict antemortem concentrations. Blood glucose concentrations are known to rapidly decrease in the hours following death; however, postmortem vitreous humor glucose concentrations are stable and can be used to determine if hyperglycemia was a factor in the decedent's death. There does not appear to be any literature investigating if burn injury changes antemortem blood glucose concentrations in post-burn injury blood specimens.

The main objective was to determine if post-burn injury blood glucose concentrations are altered by excessive thermal and/or flame burn injury compared to antemortem blood glucose concentrations.

Methodology included a Sprague Dawley rat model being used to determine the effects of burn injuries with two fire-related models, thermal and flame burn injury. The burn injuries produced by the different burn injury groups ranged from scorched hair to loss of limbs. One hundred twenty-six male rats were gavaged orally with 4g/kg of ethanol then placed in metabolic cages for three hours until carbon dioxide euthanization. Burn injuries from fire deaths were mimicked using the reported average response time by local fire departments and two types of burn injury, using a flame and thermal injury, with these conditions:

Flame burn injury (Fire Pit) (n=9 per group, 3 groups) Temperature: >1000°F	Duration (minutes): 2, 5, and 8
Thermal burn injury (Gas Grill) (n=9 per group, 9 groups) Temperature: 200°F, 400°F, or 600°F	Duration (minutes): 2, 5, or 8

Pre- and post-burn injury blood glucose concentrations were measured in the heart blood using a Relion Ultima point-of-care blood glucose monitor. Post-burn injury heart blood was collected after refrigeration of the burn injured corpses upon reaching a core body temperature of 50°F. A short duration between death and post-burn injury blood collection and refrigeration was implemented in this procedure to minimize experimental glucose changes. Pre-burn injury core body temperatures and maximum core body temperatures were measured using a rectal probe to determine if there was any correlation between core body temperatures and changes in blood glucose concentrations.

Results revealed maximum core body temperatures ranged from 90°F – 15545°F after flame or thermal burn injury. Post-burn injury blood glucose concentrations in higher maximum core body groups, flame burn injury for eight minutes, and thermal burn injury at 600°F for eight minutes were 30% and 36% greater respectively, compared to pre-burn injury blood glucose concentrations ($p<0.05$). Lesser time/temperature exposures produced a significant decrease in post-burn injury blood glucose concentration ($p<0.05$). Low maximum core body temperature groups had a 50% – 69% decrease in post-burn injury blood glucose concentrations compared to pre-burn injury concentrations. The rate of blood glucose decrease was lessened as time/ temperature exposure increased. Groups that had some of the hotter maximum core body temperatures (thermal burn injury at 400°F for eight minutes, thermal burn injury at 600°F for five minutes, and both burn injury controls) had a much smaller decrease (0% – 27%) in post-burn injury blood glucose concentrations compared to pre-burn injury concentrations.

In conclusion, the burn injury experiments using a rodent model suggest that blood glucose is altered by excessive burn injury. Despite efforts to minimize the loss of blood glucose in post-burn injury, the results indicate that at lower maximum core temperatures, post-burn injury blood glucose concentrations were significantly decreased compared to pre-burn injury concentrations. The natural decline of blood glucose was observed in lower burn injury groups. The decrease in postmortem blood glucose concentrations was disrupted as the burn injury increased in the experiments. The natural process is overshadowed by increasing blood glucose concentrations at higher maximum core body temperatures/longer exposure to burn injury. Blood glucose was significantly elevated in the Sprague Dawley rats that had excessive burn injury/higher maximum core body temperature (thermal burn injury 600°F for 8 min). The literature describes a relationship between hyperglycemia and burn patients. The severely burn-injured corpses exhibited elevated glucose concentrations and suggests that burn injury alters normal postmortem pathological changes. Time and temperature of exposure correlate to core body temperature change and result in a corresponding change in post-burn injury blood glucose concentration.

Blood Glucose, Burn Injury, Rodent Model

K12 Evaluation of the Chemical Derivatization of Nine Different Cathinone Bath Salts Analogs

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The goal of this presentation is to provide the forensic community the optimal derivative to be used in the identification and quantification of bath salts.

This presentation will impact the forensic science community by providing the optimal derivatizing agent, to date, for the drugs cathinone, methcathinone, methylone, methedrone, mephedrone, ethcathinone, ethylone, pentedrone, pentylone, and butylone to utilize in the everyday work of identifying submitted substances. Over the past few years, so-called bath salts drugs have become a major substance-abuse problem in the United States. Cathinone is a naturally occurring stimulant found in *Catha edulis*. It is a beta-keto derivative of amphetamine with similar psychological and behavioral effects. Methcathinone, methedrone, methylone, mephedrone, ethcathinone, ethylone, pentedrone, pentylone, and butylone are designer drugs of cathinone. The beta-keto phenethylamine side-chain varies in length of the alkyl chain at the gamma carbon while the phenyl ring either has no attachment, a methyl group, a methoxy group, or a methylene dioxy ring attached. While cathinone, methcathinone, mephedrone, and methylone are Schedule I drugs, the rest have not yet been controlled. Due to the structure resemblance, highly specific analytical methods such as Mass Spectrometry are necessary to accurately identify these abused bath salts.

A Multidimensional-Gas Chromatography/Mass Spectrometry (MD-GC/MS) (Shimadzu Scientific, Inc.) equipped with a Rtx-5 (20m x 0.18mm ID x 0.2df) and a Rtx-50 (10m x 0.18mm ID x 0.2df) columns (Restek Corporation), a Dean's switch, and a 2010 Ultra GC/MS system with EI ionization was used. The oven temperature was programmed from 150°C, initial hold 0.1 min, to 320°C at 25°C/min. The inlet temperature and transfer temperature were 275°C and 280°C, respectfully. The drugs were initially evaluated underivatized and then derivatized using 5µg of standard in methanolic solution and evaporated to dryness under N_2 at room temperature. The residue was derivatized using either Heptafluorofutyric Anhydride (HFBA), Propionic Anhydride (PA), Acetic Anhydride (AA), Trimethylsilane (TMS, BSTFA + 10% TMCS), t-butyl (MTBSTFA, MTBSTFA + 1% TBDMCS), MethElute, or N-trifluoroacetyl-*l*-triprolyl chloride (*l*-TPC).

The underivatized bath salts analysis resulted in poor chromatographic characteristics and small base peak fragment ions. The optimal derivative was determined to be HFBA, which demonstrated the best chromatographic resolution, characteristic fragmentation, and symmetrical peak shape. HFBA required a lower temperature and shorter reaction time to obtain the derivatives as compared to PA, AA, TMS, and MTBSTFA. Flash derivatization was performed with MethElute. The HFBA derivative results are as follows:

Drug	R_t (min)	Base Ion (m/z)	Qualifier ion m/z (relative abund.%)	Molecular Weight (m/z)
Cathinone	4.5	105	240 (30), 77(4)	345
Methcathinone	4.6	105	254 (29), 77 (81)	359
Ethcathinone	5.0	268	373 (0.2), 134 (2), 105 (50), 77 (21)	373
Mephedrone-D3	5.1	119	257 (21), 91(18)	376
Mephedrone	5.2	119	373 (0.1), 254 (20), 91 (20)	373
Pentedrone	5.2	282	387 (1), 105 (61), 77 (26)	387
Methedrone	5.9	135	254 (8), 107 (6)	389
Methylone	6.4	149	254 (18), 121 (11)	403
Methylone-D3	6.4	149	406 (3), 257(19), 121 (11)	406
Butylone	6.6	149	417 (4), 268 (27), 121 (11)	417
Ethylone	6.7	149	417 (2), 268 (33), 121 (11)	417
Pentylone	6.9	149	431 (3), 282 (21), 121 (10)	431

The shift in retention times of the PA and AA derivatives showed successful derivatization, but the mass spectrum contained uncharacteristic 44m/z and 58m/z base ions. The MTBSTFA, TMS, and MethElute derivatives revealed incomplete derivatization resulting in up to two peaks; a derivatized drug peak and underivatized drug peak or only the completely underivatized. The *l*-TPC derivative was successfully synthesized, resulting in two peaks in mixtures where the solution contained a racemic mixture of the drug.

The HFBA derivative was determined to be the best overall derivative for the identification of the listed bath salts. Bath salts can also be derivatized using *l*-TPC, which can assist in differentiating steroisomers if needed.

Bath Salts, Derivatization, Mass Spectroscopy

K13 "Benzofury" Also Known as 6-APB (6-(2-Aminopropyl)-2,3-Dihydrobenzofuran): A Recent Fatality Involving an Unusual Drug

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After attending this presentation, attendees will be alerted to a popular new "research chemical" being sold over the internet, 6-(2-aminopropyl)-2,3-dihydrobenzofuran or 6-APB, commonly referred to as "Benzofury" and being used as an ecstasy substitute. This presentation will aid forensic toxicologists in the identification of this unusual compound and provide quantitation data from a recent postmortem case.

This presentation will impact the forensic science community by providing scientific literature from a recent postmortem case involving 6-APB. There is little to no toxicological data available at this time and the presentation of this case may help in compiling such data.

Benzofury (6-(2-Aminopropyl)-2,3-dihydrobenzofuran) or 6-APB, has become a popular "research chemical" available for sale over the internet. It is being marketed as "plant food" and has recently become available in its pure powder form. It is gaining popularity among recreational users for its reported euphoric and psychedelic properties, which are thought to be similar to Methylenedioxyamphetamine (MDA). It is in the phenethylamine class of drugs and an analog of 3,4- MDA, with an oxygen atom within the methylenedioxy portion of MDA being replaced with a methylene group. It is thought to be an entactogen, stimulant, and psychedelic drug and is currently unscheduled in the United States. However, it may be covered in the Federal Analog Act and is still currently legal in several other countries. Dr. David Nichols and his team first investigated 6-APB around 1993 at Purdue as a potential antidepressant which could also possibly be used in conjunction with psychotherapy.

A 21-year-old male had been drinking and using drugs over the course of an evening with two friends at a local motel in Peoria, AZ. They purchased nine pills of what they thought were ecstasy, each consuming three of the pills. During the night, the decedent became aggressive and violent and the friends feared they would be removed from the property. One of the friends put the decedent face down on the bed and straddled him in an effort to calm him. The attempt to restrain him lasted approximately 15 minutes and ended with the friend putting the decedent in a choke hold. The decedent was then found to be unresponsive and emergency medical services were summoned. He was transported to a local hospital where his death was pronounced in the emergency department.

The decedent was transported to the medical examiner's office where a full autopsy was performed. The only notable findings were contusions from the restraint. Multiple postmortem samples including vitreous, cardiac blood, urine, bile, and gastric contents were collected and sent to the toxicology laboratory for testing. Blood and urine specimens were subjected to a qualitative analysis using a basic pH screen with a liquid/liquid extraction and analyzed by Gas Chromatography/Nitrogen Phosphorus Detection (GC/NPD), then confirmed by Gas Chromatography/Mass Spectrometry (GC/MS). Volatiles were assayed on vitreous and cardiac blood using Gas Chromatograph/Flame Ionization Detector (GC/FID). The blood was also screened by Enzyme-Linked Immuno-Sorbent Assay (ELISA) for barbiturates, benzodiazepines, benzoylecgonine, opiates, methamphetamine, and fentanyl. The methamphetamine screen reacted at a low positive level. A sympathomimetic amine quantitation was performed on the cardiac blood by GC/MS Selected Ion Monitoring (SIM), with a large unidentified peak seen on the Thermal Imaging Camera (TIC). A significant peak was also seen on the GC/NPD screen as well as the GC/MS/TIC, which also was unidentified. The peak was subsequently identified as 6-APB and a known standard was obtained courtesy of the DEA Special Testing Laboratory. The 6-APB was quantitated using a sympathomimetic amine method and the concentration was determined by comparing the peak area ratios of 6-APB to the internal standard (MDA-D5) against a standard curve, with linearity demonstrated up to 1.0mg/L. Fractional volumes were used for samples exceeding linearity. The concentration of 6-APB in the decedent's cardiac blood was found to be 2.15mg/L. Ethanol was found in the decedent's cardiac blood and vitreous at 0.05mg% and 0.09 mg%, respectively.

The cause of death was listed as external compression of the neck and the manner of death was homicide. Benzofury (6-APB) was listed a contributing factor. In its pure powder form, 6-APB is usually ingested orally with the onset of effects reported within 30 – 90 min after ingestion. Scant information regarding this drug is available; therefore, little is known about its dosing and toxicity.

Benzofury, MDA, Postmortem

K14 Phenazepam and Driving Impairment: A Case Report

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After attending this presentation, attendees will understand the potential for phenazepam, a lesser known 1,4-benzodiazepine to impair driving performance.

This presentation will impact the forensic science community by providing an increased understanding of phenazepam impairment, specifically the extent to which it may impair some individuals at low dose.

Phenazepam is a 1,4-benzodiazepine that is structurally related to lorazepam and bromazepam. It originated in the Soviet Union in the 1970s and recently emerged as a drug of abuse. It is reported to be one of the most frequently prescribed benzodiazepines in Russia and other Commonwealth of Independent State (CIS) countries. Although it has no legitimate clinical uses in the United States, it has been used therapeutically for its sedative hypnotic, anticonvulsant, muscle relaxant, anxiolytic, and for the treatment of alcohol withdrawal overseas. When used therapeutically, it is available as 0.5mg and 1mg tablets, injectable solutions (0.1% and 0.3%) and transdermal patches. Oral doses of 0.5mg (2 – 3 times daily) may be prescribed, but doses up to 10mg/day are reported.

Several eastern European countries have taken steps to control phenazepam. Here in the United States, it is not controlled at the Federal level, although two states (Arkansas and Louisiana) enacted recent legislation to control the drug. Illicitly, it is available as a powder, tablet, and blotters (similar to LSD). Recreational users report doses of 2mg – 10mg of the drug. There have been relatively few pharmacological or toxicological studies involving phenazepam. In one study involving doses of 3mg – 5mg, peak plasma concentrations of 24 ng/mL – 38ng/mL were observed at approximately 4 h with a half-life of approximately 60 h. When 2mg doses were administered intramuscularly in epileptic patients, the half life was estimated to be 15 h. Adverse effects may include somnolence, dizziness, incoordination, and asthenia.

In a report from Finland, 3.4% of all Driving Under the Influence of Drug (DUID) cases were found to contain phenazepam. In the vast majority of cases (77 of 83 positive cases), other drugs were also detected. Multiple drug use can complicate interpretation, particularly for drugs that are less studied. Performance deficits attributed to phenazepam include unstable gait, confusion, impaired balance, slurred speech, memory loss, ataxia, and pupils that are slow to react to light.

A case is reported of a 24-year-old male apprehended for impaired driving. The subject failed to stop at an intersection and was involved in a two-vehicle crash. The subject had slurred speech and profound psychomotor impairment. His balance was poor; he

staggered, and after being placed in a chair, was unable to stand without falling. Blood toxicology was initially negative at another laboratory. The sample was sent to SHSU Regional Crime Lab for additional testing due to the inconsistent results. Comprehensive toxicology testing by Solid Phase Extraction (SPE) and Gas Chromatography/Mass Spectrometry (GC/MS) revealed the presence of phenazepam at a concentration of 76ng/mL in blood. No other drugs were detected. Phenazepam was quantitated using an Agilent HP 5975 MSD/6890 GC with a HP-5MS capillary column (30m x 0.25mm x 0.25µm). In the absence of deuterated phenazepam, prazepam was used as the internal standard.

The immunoassay cross-reactivity of phenazepam was investigated and found to be >250% using the immunalysis benzodiazepine Enzyme Linked Immuno-Sorbent Assay (ELISA) used in the laboratory. Due to the high cross-reactivity, the sample screened presumptively positive at the 50ng/mL (oxazepam) cutoff. Initial screening at the first laboratory by Enzyme Multiplied Immunoassay Technique (EMIT) was negative, resulting in no further testing. This case report highlights the importance of cross-reactivity in immunoassay and the need to perform more extensive, broad spectrum screening for impaired driving cases, especially when impairment and toxicology results are inconsistent. In this case report, severe impairment was observed in an individual following phenazepam use. The concentration detected was consistent with a single dose of the drug. Phenazepam is a lesser known low-dose benzodiazepine with the potential for significant traffic safety consequences.

Phenazepam, Impairment, Immunoassay

K15 Identification and Quantification of Tapentadol and N-Desmethyltapentadol in Human Urine Using Gas Chromatography-Mass Spectrometry

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After attending this presentation, attendees will learn about a Gas Chromatography-Mass Spectrometry (GC/MS) method developed to identify and quantify tapentadol and its main metabolite N-desmethyltapentadol (NDT) in human urine. Attendees will also understand that preliminary investigations demonstrated that the NDT metabolite does not have the same extraction characteristics and chemical derivatization properties as the parent drug. Therefore, special considerations were necessary when developing a method for simultaneous identification and quantification of tapentadol and NDT in human urine.

This presentation will impact the forensic science community by describing to forensic toxicologists and chemists the first GC/MS method developed and validated to identify and quantify tapentadol and its main metabolite NDT in human urine.

Chronic pain is one of the most persistent health care problems in the United States. When physicians fail to properly address pain in patients, it can lead to additional health problems or decrease the patient's quality of life. In many health care settings, opiate and opioid drugs have become the treatment of choice for pain management because of their effective analgesic properties. Tapentadol (Nucynta®) is a relatively new drug that is approved for treatment of both immediate and chronic pain. Tapentadol causes analgesia by acting as an agonist at the brain's mu receptors and as a norepinephrine reuptake inhibitor. Combining these two mechanisms of action makes tapentadol different from "traditional" opiate and opioid drugs, which do not act as norepinephrine reuptake inhibitors. As an analgesic drug, it is likely that incorporation of tapentadol into pain management and pain-monitoring programs will become more widespread, and it will be necessary for toxicology laboratories to be able to identify and quantify the drug and its metabolite in human urine specimens.

The development and validation of the first GC/MS assay developed for the identification and quantification of tapentadol and its major metabolite NDT in human urine samples will be described. Method development studies were initially performed to design an assay that was optimized for the extraction, identification, and quantification of tapentadol and NDT. The optimized procedure involved sample alkalinization with saturated borate buffer (pH 9.5) and extraction into chloroform: isopropanol (9:1) solvent. Samples were evaporated to dryness under a stream of nitrogen and derivatized with 25µl MTBSTFA + 1% TBDMCS: acetonitrile (1:2) at 55°C for >2h.

Quantification of tapentadol and NDT required two internal standards. Deuterated tapentadol-d3 was used for tapentadol quantification and 4-(2-methylamino)propyl)phenol was used for NDT because deuterated NDT was not commercially available. Two internal standards were needed because of the extraction differences between tapentadol and NDT, arising from the metabolite's secondary amine structure. The secondary amine structure of NDT also limited the compound's solubility in MTBSTFA + 1% TBDMCS derivatizing reagent, making it necessary to solubilize the metabolite with acetonitrile to maximize silation derivatization.

Glucuronide conjugates are the primary route of tapentadol and NDT elimination. Hydrolysis studies were performed to liberate the glucuronide conjugates from urine samples. Tapentadol-β-D-glucuronide and NDT were analyzed using the developed GC/MS program and no free tapentadol was detected. Overnight hydrolysis with helix pomatia H-2 was found to be the optimal hydrolysis method, with approximately 50% tapentadol liberated at concentrations of 150ng/ml, and 47% at concentrations of 600ng/ml.

The assay met all laboratory validation criteria with respect to linearity, sensitivity, accuracy, inter-assay precision, intra-assay precision, selectivity, matrix effects, process efficiency, recovery, bench-top stability, and instrument stability. The Limit of Detection (LOD) and Limit of Quantification (LOQ) for tapentadol and NDT were administratively set at 10ng/ml and 50ng/ml, respectively.

Five quality-control samples were run in triplicate over an eight-day validation period with tapentadol and NDT at concentrations of 50, 150, 600, 1500, and 2500ng/ml. The accuracy of the quality-control samples were within ±16% of the target value and the precision %CV values (inter and intra) were <16%. Matrix effect, process efficiency, and recovery were assessed by analyzing six replicates of tapentadol and NDT at concentrations of 150 and 600ng/ml. At both concentrations, tapentadol recovery was 100% and NDT recovery was 83% at 150ng/ml and 96% at 600ng/ml. The validated method allows for the identification and quantification of tapentadol and NDT in human urine.

Tapentadol, N-Desmethyltapentado, GC/MS

K16 Deaths Involving the Recreational Use of α-PVP (α-pyrrolidinopentiophenone)

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After attending this presentation, attendees will gain a better understanding of potentially fatal consequences involving the stimulant hallucinogen α-pyrrolidinopentiophenone (α-PVP). Attendees will also obtain information addressing the measurement of this compound in human autopsy samples.

This presentation will impact the forensic science community by providing useful information regarding the toxicological analysis of cases involving designer stimulants, highlighting the need to consider the potential involvement of such drugs when presented with suggestive incident circumstances.

Until recently, the most common stimulant hallucinogen used illicitly in the United States was MDMA. However, the rapidly escalating availability of a variety of highly potent analogues has resulted in drug-related morbidity and mortality including violent confrontations, motor vehicle accidents, suicides, and fatal drug toxicity. A group of these compounds, collectively referred to as "bath salts," have been sold via the internet and through a variety of retailers including gas stations, convenience stores, and so-called "head shops." Typically, the compound present in these preparations has been 3,4-methylenedioxypyrovalerone (MDPV). Over a three-month period beginning in March 2012, the West Virginia Office of the Chief Medical Examiner has investigated three deaths involving a similar drug, α- α-PVP. The mechanism of action of this drug is thought to involve inhibition of the reuptake of norepinephrine, dopamine, and serotonin. Here is reported case circumstances and toxicological findings in three deaths involving α-PVP in which the decedents exhibited aggression, paranoia, violence, and homicidal behaviors.

Samples obtained at autopsy underwent routine postmortem toxicological testing. This included blood alcohol analysis by Gas Chromatograph/Flame Ionization Detector (GC/FID), drugs of abuse by immunoassay, Liquid Chromatography/Time Of Flight/Mass Spectrometry (LC/TOF/MS) screening of blood precipitates, and Gas Chromatograph/Mass Spectrometry (GC/MS) screening of alkaline and acidic/neutral blood extracts. GC/MS analysis of alkaline extraction of urine utilizing Toxi tube A was helpful in identifying the presence of α-PVP. Confirmation and quantitation of α-PVP was performed by GC/MS analysis of an alkaline liquid-liquid extract (without derivatization).

The decedents were adult males aged 31, 35, and 51 years. The oldest male was found deceased on his bathroom floor. Empty packages of bath salts were discovered at the scene with yellowish-tan powder noted within the nostrils. The most significant toxicological finding was α-PVP at a concentration of 0.10mg/L in the blood. THC and carboxy-THC were also present at 2.6 and 25ng/mL, respectively. The second fatality involved witnessed seizure activity preceding the demise. A history of bath salt and prescription drug abuse was reported. Toxicology results included α-PVP at a concentration of 0.52mg/L in the blood in addition to sertraline (0.16mg/L), oxycodone (0.02mg/L), and 7-aminoclonazepam (<0.01mg/L). The youngest male died from firearm injuries during an armed confrontation with law enforcement involving aggressive and paranoid behavior, as well as suicidal threats. Vials believed to contain bath salts were found in the decedent's pockets. Drugs confirmed in the blood included α-PVP and pentedrone at concentrations of 0.29 and 0.48mg/L, respectively. In all three cases, α-PVP was deemed to be the primary cause of death or a significant contributory factor.

Current routine postmortem toxicological analysis may not detect many of the designer stimulant compounds that present an increasing challenge in forensic pathology and toxicology. Often, cases in which the history documents bizarre, aggressive, hallucinogenic, or paranoid behaviors, and/or symptoms consistent with overstimulation of the sympathetic nervous system are positive for compounds such as α-PVP or other cathinone derivatives upon targeted analysis. Similar to other sympathomimetic drugs, establishing toxic and lethal concentrations for α-PVP will likely be difficult. These drugs often demonstrate significant overlap between concentrations tolerated by individuals and those reported in drug-related fatalities. Investigative history, autopsy findings, and toxicology results must be fully assessed to most accurately determine the cause and manner of death in cases involving designer stimulants such as α-PVP.

α-PVP, Bath Salts, Postmortem

K17 Two Cases of Suicide in Nurses by Atracurium: Revealed by LC/ESI/MS

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The goal of this presentation is to show two suicidal cases of atracurium in nurses, revealed by Liquid Chromatography/Electrospray Ionization/Mass Spectrometry (LC/ESI/MS). The analytical method and the postmortem toxicological concentrations of atracurium and laudanosine revealed in both fluids and tissues are discussed.

This presentation will impact the forensic science community by showing the importance of an analytical method developed for simultaneously quantifying postmortem of atracurium and its metabolite laudanosine in two suicidal cases.

Atracurium is a non-depolarizing skeletal muscle relaxant. It is a derivative of curare, a plant extract prepared from many different plants of the Amazon forest, used by the natives of the area as a poison arrow for hunting and war. It is used to facilitate endotracheal intubations and to relax skeletal muscles during surgery or mechanical ventilation. It is available as a 1% solution of the besylate salt for intravenous administration. It can be fatal in any concentration due to respiratory failure, so controlled ventilation is necessary. Following an intravenous dose, the muscles begin to relax within about two minutes and the effect lasts for 15 min – 35 min, depending on the dose. The drug is excreted in urine and bile, and its elimination half-life is around 20 min.

This presentation concerns two lethal cases of polydrug intoxication, both positive for the atracurium:

- The first case (named "A") involved a nurse of the Emergency Unit found dead in his home. Near his body, a syringe containing few cc's of colorless liquid and an empty blister pack of tablets of midazolam were found.
- The second case (named "B") involved to a nurse found unresponsive in the hospital where he was employed. Near his body, a syringe containing 11cc's of colorless liquid and an empty bottle showing the words "sodium pentothal" were found.

A comprehensive toxicological screening was performed on postmortem cardiac blood, urine, bile, and tissue homogenates (liver, heart, and kidney) using a combination of immunoassay and chromatographic techniques.

In detail, in both cases, lethal concentrations of midazolam were confirmed in biological fluids and tissues of the body A, while the presence of thiopental was revealed in biological fluids and tissues of the body B.

Since atracurium degradation occurs rapidly *in vitro* by the same hydrolysis mechanism observed *in vivo*, and it is accelerated by an alkaline pH and high temperatures, and given its simultaneously precharged yet lipophilic nature, detecting low atracurium levels in human postmortem samples is a challenge.

A method was developed for simultaneously quantifying low levels of atracurium and its less polar metabolite laudanosine in postmortem blood, bile, urine, and tissues by LC/MS in an ion trap mass spectrometer under positive ion ESI conditions. Analytes were isolated from blood and tissues by solid-phase extraction using Bond-Elut Certify columns. The method proved selective and sensitive, and was validated in postmortem blood, bile, urine, heart, kidney, and liver in the range of 1 – 2000ng/mL (blood) and 5 – 5000ng/g (tissues). The proposed method was fully validated with respect to previously published LC/MS methods.

Lethal concentrations of atracurium and laudanosine were confirmed in all the biological fluids and tissues of both bodies. The presence of atracurium was also confirmed by toxicological examination of the colorless liquid found in syringes.

Based on the autopsy findings, case history, and toxicology results, the forensic pathologists ruled that the cause of death in both cases was an overdose of atracurium in combination with midazolam for body A and thiopental for body B; the manner of death was suicide.

Atracurium and Laudanosine, Liquid Chromatography, Toxicological Finding

K18 Direct Analysis in Real Time (DART®) Analysis With a Modified GC/MS System for Rapid Drug Screening

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After attending this presentation, attendees will learn about complete rapid screening for drugs of abuse using Direct Analysis In Real Time (DART®) screening capability with their Gas Chromatograph/Mass Spectrometry (GC/MS). This is important since traditionally the DART® technology has required a more complex Liquid Chromatography/Mass Spectrometry (LC/MS) for operation.

This presentation will impact the forensic science community by enabling more rapid screening of samples with existing GC/MS systems that are prevalent in the community. This technology can speed identification of drugs of abuse, reduce turn-around-time, and reduce sample backlogs.

DART® is an ambient ionization method that provides rapid determination of sample composition with little sample preparation. Samples are directly sampled and ionized merely by placing the material in the flow of heated ionizing gas. Solids or liquids are readily analyzed, often without any manipulation or purification of the sample. The ionization usually occurs by the excited helium atoms reacting with ambient water to form protonated water clusters. These water clusters attach a proton onto the molecule of interest, producing a spectrum that is very simple and often composed of one peak per compound. This leads to the facile interpretation of the spectra and the ability to analyze mixtures without the complexity of many fragment ions.

Several major forensics laboratories including FBI, Secret Service, the FDA Forensic Chemistry Center, and the Virginia Department of Forensic Science, have utilized DART® for rapid detection and characterization of unknowns. Published papers show analysis of gamma-hydroxybutyric acid, synthetic cannabinoids, analysis of sexual assault evidence, alprazolam tablets, methamphetamine, bank security device and pepper spray components, explosives trace detection, ricin activity assay, iodine and red phosphorus, and chemical warfare agents.[1-10]

However, this time-saving ambient ionization technology has not gained a wider audience in the forensic community for several reasons. A major reason is the fact that the DART® source requires a mass spectrometer equipped with an Atmospheric Pressure Inlet (API) typically supplied with an LC/MS system. Since this technology is limited to more specialized laboratories, the analysts cannot readily access the current DART® technology. In this current effort, an API has been integrated into an Agilent Mass Selective Detector (MSD), which is widely used in state and federal laboratories for trace forensic analysis. Secondly, the sampling process has been left to the analyst, allowing for flexibility, but also encumbering the analysts with additional method development. The sample preparation process has been simplified with a new device composed of a card-containing metal screen that holds the liquid or solid sample. The sample is placed on the screen, the card is inserted into the source, and the spectrum acquired in less than 10 seconds.

Facilitating DART® analysis with the low cost Agilent mass analyzer should enable more laboratories to add this capability, speeding analysis and reducing backlogs. This presentation will demonstrate the application of this modified DART®-MSD for determination of the presence of drugs in urine with a simple solid phase extraction for sample preparation. This reduces analysis time of 30 – 60 min to less than one minute. Additionally, the direct analysis of solid dosage forms of drugs of abuse will be illustrated, showing how DART® can identify these materials in seconds.

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Drugs of Abuse, DART®, Mass Spectrometry

K19 Survey of Practices in Toxicological Investigation of Drug-Impaired Driving

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After attending this presentation, attendees will be able to compare their laboratory's practices with peer laboratories and evaluate typical cutoffs used for drug screening and confirmation. This presentation will describe data from a survey carried out to evaluate the practices of forensic toxicology laboratories performing analysis in the investigation of Driving Under the Influence of Drugs (DUID) cases. The survey was sponsored by the National Safety Council's Committee on Alcohol and Other Drugs (NSC CAOD).

This presentation will impact the forensic science community by providing data to support updating of general recommendations for laboratory testing in DUID investigations in order to improve consistency and standards of screening and confirmation.

The purpose of this survey was to evaluate scope and sensitivity of testing, compliance with the current recommendations for DUID testing, and changes in patterns of drug use by drivers in DUID investigations that might warrant updating of previous recommendations. This research aimed to assist in critically reviewing and updating the current guidelines and recommendations for the toxicology community.

An online web-survey instrument was used. The survey questions focused on scope and sensitivity for drug screening and confirmation, analytical methods, and ability to meet previously published recommendations.[1] The final revised survey was sent to confirmed participants via the online survey. Follow-up emails and phone calls were used to obtain additional information or clarify responses. In spite of these efforts, some participants did not respond to all questions; therefore, the data represents 96 surveys completed to the point where they were deemed sufficiently complete for inclusion in the data analysis.

It was indicated that 80% of responding labs test blood samples and 68% reported testing urine samples in DUID casework. Few labs reported testing oral fluid, and not consistently. Screening methods for blood testing were mostly Enzyme-Linked Immuno-Sorbent Assay (ELISA) (34%), Gas Chromatograph/Mass Spectrometry (GC/MS) (28%), Liquid Chromatography/Mass Spectrometry (LC/MS) (17%), and Enzyme Multiplied Immunoassay Technique (EMIT) (13%). No labs reported using Liquid Chromatography Time-Of-Flight (LCTOF) screening for blood. For urine, 29% reported GC/MS screening, ELISA (27%), EMIT (23%), and LC/MS (14%). For confirmatory testing, 52% of labs reported using GC/MS, while 36% used LC/MS. Labs were asked about reporting unconfirmed results, and 33% indicated they would report those under some circumstances, including insufficient sample, lack of a confirmatory procedure (with a recommendation to have testing sent out), and emphasized the inclusion of disclaimer about the presumptive nature of the result.

Respondents were asked whether their laboratories practices were consistent with the 2007 recommendations. Responses varied by drug and matrix. For screening purposes, the majority of labs reported meeting or exceeding the guideline recommendations for drugs of abuse, including carboxyTHC, benzoylecgonine, benzodiazepines, MDA, barbiturates, methadone, opiates, and PCP. The majority did not meet the recommendations for amphetamines. Drugs for which the majority of laboratories did not meet the recommendations for confirmatory testing were mostly therapeutic drugs including trazodone, nortriptyline, carisoprodol, zolpidem, topiramate, and methadone.

Participants were asked to indicate which additional drugs should be included in the recommendations for routine screening and confirmation. At least 75% of the 68 participants who responded to this question indicated that mephedrone, zopiclone, and buprenorphine should be included in future recommendations for blood sample screening. Additionally, at least 50% of the participants indicated that methylone, MDPV, JWH-073, JWH-250, JWH-081, JWH-122, JWH-210, JWH-019, JWH-200, AM-2201, benzylpiperazine, trifluromethylphenylpiperazine, dimethyltryptamine, modafinil, quetiapine, and zaleplon should be included in the future recommendations for blood sample screening.

Based on this input, the NSC CAOD is updating the guidelines for distribution early in 2013.

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DUID, Cutoffs, Guidelines

K20 Simultaneous Quantification of Amphetamines, Ketamine, and Opiates in Urine Using SPE and LC/MS/MS

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After attending this presentation, attendees will learn of a Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) technique for analyzing amphetamine, methamphetamine, MDA, MDMA, morphine, 6-acetylmorphine, codeine, 6-acetylcodeine, ketamine, and norketamine in urine.

This presentation will impact the forensic science community by developing a simple, accurate, and fast analytical method of LC/MS/MS capable of quantifying ten analytes in urine that are abused drugs.

Heroin, methamphetamine, and ketamine have historically been the most commonly abused drugs in Taiwan and are routinely monitored in the laboratory by Gas Chromatography/Mass Spectrometry (GC/MS) methods. The purpose of this study was to evaluate whether the LC/MS/MS-based approach can be more effectively applied to the simultaneous quantitation of amphetamine (AM), methamphetamine (MA), MDA, MDMA, morphine (MOR), codeine (COD), 6-acetylmorphine (6-AM), 6-acetylcodeine (6-AC), ketamine (K), and norketamine (NK) in postmortem urine specimens.

Samples (1mL) were extracted via solid-phase extraction, evaporated, and reconstituted in the mobile phase for injection onto the LC/MS/MS system. Deuterated analogues of the analytes of interest were used as internal standards. Chromatographic separation was achieved using an Agilent Zorbax SB-Aq (100mm ´ 2.1mm i.d., 1.8-□m particle) analytical column at 50°C. The mobile phase consisted of 0.1% formic acid (v/v) in water (A) and methanol (B) at a flow rate of 0.32mL/min. The initial gradient composition (A/B 90:10, v/v) was held for 1.5 min, then decreased to 0% A in 8.5 min and held for 2 min, then increased to 90% A in 1 min and held for 2 min. MS analysis was performed by an electrospray ionization in positive-ion Multiple Reaction Monitoring mode (MRM) with optimized collision energy for the precursor ion selected, monitoring two transitions for each analyte.

Validation was performed by extracting drug-free urine fortified with 50 – 1000ng/mL of the 10 analytes, yielding the following results: (1) average extraction recovery (n=5) was >80%, except for MDMA (70%) and MOR (74%); (2) inter-day and intra-day precision ranges (%CV) were 1.59 – 9.13% and 0.57 – 3.89%; (3) calibration linearity (r^2), detection limit, and quantitation limit were >0.997, 1ng/mL and 5ng/mL for all analytes, respectively; and, (4) matrix effects: ion suppression was lower than 20% for all analytes; it was compensated by using deuterated internal standard. Compared with traditional GC/MS methods, the conclusion arose that this relatively simple protocol can be used for routine and reliable identification and quantitation of AM, MA, MDA, MDMA, MOR, COD, 6-AM, 6-AC, K, and NK in urine. This method was successfully applied to the analysis of postmortem and antemortem specimens from forensic cases.

Drugs of Abuse, Urine, LC/MS/MS

K21 Fatal Cases of Aconitum Alkaloids Poisoning in Korea

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After attending this presentation, attendees will gain knowledge regarding concentration levels in fatal cases of aconite poisoning and detection methods.

This presentation will impact the forensic science community by showing how this method was successfully applied to detect aconitines in various specimens of related aconitum alkaloids poisoning cases.

Aconitum alkaloids have been occasionally used in Korean herbal medicine because of pharmacological effects such as analgesic, anti-epileptic, and anti-inflammatory, but they can lead to sudden death by their cardiotoxins and neurotoxins. In traditional medicine, aconite roots are used only after processing to reduce the toxic alkaloid content. Soaking and boiling during processing will hydrolyze aconite alkaloids into less toxic and non-toxic derivatives; however, the use of a larger-than-recommended dose and inadequate processing increases the risk of poisoning. Every year, several causes of death were contributed to aconite toxicity. The high levels of toxicity of aconite are considered to be derived from aconitine, mesaconitine, and hypaconitine. The lethal dose of aconitine in human adults is estimated to be only 1mg – 4mg.

There have been reported cases of homicide, suicide, and accidental ingestion. Severe aconite poisoning can occur after accidental ingestion of wild plants or consumption of herbal decoction-made aconite roots. Some fatal cases were caused by unrefined herbal medicine prepared from aconite. Aconitum alkaloids have been indentified in various samples such as traditional prescribed herbal medicine, aconite infusion water, aconite liquor, wild greens mixed aconite, and their biological specimens from five cases related to aconite poisoning this year.

A rapid, specific, and sensitive Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method was developed for simultaneous identification of aconitine, mesaconitine, and hypaconitine. The determination of aconitum alkaloids in specimens was performed by LC/MS/MS after liquid-liquid extraction using trimipramine-d3 as an internal standard. Samples of each, 1mL or 1g, were extracted with 5mL of ethyl acetate in alkali of NH4OH. The organic layer was dried with a stream of nitrogen at 45°C. The residues were reconstituted with methanol and injected into LC/MS/MS. The separation was applied on Agilent XDB C18 column (1.8 micron, 4.6×50 mm). The injection volume was 5µL and the retention time was less than 8 minutes. A gradient elution of acetonitrile and water of 0.1M ammonium formate and 0.1% formic acid were used as mobile phase. Flow rate was 0.4mL/min. LC/MS/MS system (ABSciex, ABI 3200QTrap) coupled with an Electrospray Ionization (ESI) source was performed in multiple reaction monitoring(MRM) mode. The transitions of the Aconitum alkaloids executed as follows: m/z 646.3→586.0 for aconitine, m/z 632.3→572.4 for mesaconitine, m/z 616.3→556.3 for hypaconitine, and m/z 298.3→103.0 for trimipramine-d3 as internal standard. This method was successfully applied to detect aconitines in specimens. The validation results of selectivity, matrix effect, recovery, linearity, intra- and inter-assay precision, and accuracy were satisfactory.

It is well known that aconite poisoning can cause various symptoms, including arrhythmia and death, but specific autopsy findings are not configurative. There is a potential risk to overlook the death by aconite ingestion without advance information. When given more information about the scene, this method is useful to investigate aconite poisoning. At the same time, the public should be warned of the danger of eating wild plants and be educated on the potential hazards from self treatment with aconite root. In addition, it is necessary to have institutional restrictions on aconite medicine.

Aconitine, Fatality, LC/MS/MS

K22 Flight Activity and Drug Use: Legislation and Toxicological Statistics From 2006 – 2012 at the Rome Medical Legal Institute of the Italian Air Force

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After attending this presentation, attendees will understand the medical assessment of commercial pilots and cabin crews, which has two main purposes: (1) to assess their functional ability; and, (2) to ascertain whether they are physically able to safely exercise the privileges of their licenses and to verify the risk of incapacitation during the period of validity of the medical certificate.

This presentation will impact the forensic science community by demonstrating the importance of continuous surveillance of commercial pilots and cabin crews. As this study confirms, the percentage of drug users in this category of workers is very low.

The goal of this presentation is to describe the experience of the Italian Air Force Medicolegal Institute of Rome and the Forensic Laboratory of the Catholic University regarding the medical assessment of commercial pilots and cabin crews.

Materials and Methods: The total number of Class 1 and 2 medical examinations undertaken during a six-year period from January 2006 to the first semester of 2012 was taken from the Italian Air Force Medicolegal Institute of Rome medical records database.

The normative references in the relevant period regarding personal fitness to fly are: Italian Presidential Decree n. 566 November 18, 1988; JAA JAR-FCL 3 Flight Crew Licensing (medical) Amendment 5, December 1, 2006.

Urinary screening for the qualitative detection of drugs was carried out using the immunochemical technique Kinetic Interaction of Microparticles in a Solution (KIMS). The following substances were tested for: amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates. All samples were processed to guarantee chain of custody, obliging operators to document the different stages of the sample. Samples were separated into two aliquots (sample and counter-sample) and closed in front of the patient with a tamper-proof seal signed by the healthcare operator and the patient. The counter sample of urine that tested positive in a preliminary analysis was kept in the freezer at -20°C for 60 days, to be used in case any medical-legal disputes arose.

Results: The results of preliminary analysis of the urinary specimens were then examined and elaborated. Data review allowed the evaluation of the sample distribution by gender, age, drug substance type, with subsequent confirmation by Gas Chromatography/Mass Spectrometry (GC/MS). Within the positive samples, analyzed using the KIMS, the gender distribution is almost equal (five male subjects, compared to four female subjects) with an age range of 19 – 50 years.

In the relevant period, of 7,530 subjects tested for drug use (only extraordinary medical examination), nine were positive to KIMS screening. Among the positive subjects, none were polydrug users. Distribution of positive results for drugs indicated a clear prevalence of cannabinoids (eight subjects, or 89%). Only one positive case was detected for cocaine (11%) and no samples were positive for barbiturates, benzodiazepines, amphetamines, or opiates.

The cases that were positive after urinary screening, and their samples, were then subjected to confirmation by GC/MS. Of the nine positive cases, five cases (equal to 56% of all positive) haven't been confirmed. In the four confirmed cases, one was detected for cocaine and three for cannabinoids.

Discussion and Conclusion: Thanks to the continuous surveillance of commercial pilots and cabin crews, the study shows that the percentage of drug users is very low; therefore, this result indicates that it is appropriate to continue this strict type of monitoring. This phenomenon should not be underestimated since it can influence the ability of individuals who are responsible for the safety of others.

Substances of Abuse, Italian Air Force, Toxicological Investigation

K23 Blood Transfusions and Their Influence on the Evaluation of Postmortem Alcohol Levels in Biological Fluids in Road Traffic Accidents: Case Report And Review of Literature

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After attending this presentation, attendees will understand the relationship between blood transfusions and postmortem alcohol levels in biological fluids.

This presentation will impact the forensic science community by discussing drunk driving and its social consequences.

Introduction: Traffic accidents are an important cause of death, particularly among young people. To drive under the influence of alcohol and/or drugs is the cause of many of these accidents. In particular, the alcohol concentrations are measured in a corpse through the toxicological postmortem analysis on the body fluids. The blood represent the biological liquid that allows verification of drunk driving at the time of the accident. In particular, the concentration of alcohol in the blood is subject to variations due to different etiological factors. Blood alcohol levels in the corpse are altered by the putrefactive phenomena. Also, blood transfusions may influence the concentration of blood alcohol levels. This medical practice is performed to restore hemodynamic parameters in patients with critical conditions, usually after a traffic accident. The exact determination of blood alcohol levels is very difficult to determine during the autopsy examination of the corpse which has been exposed to the blood transfusion procedure.

Objective: The goal of this study is to examine the influence of blood transfusion on blood alcohol levels in cases of deaths from traffic accidents. In particular, the focus is on the reliability of postmortem toxicology data in subjects during the last stages of life who have been transfused to be resuscitated.

Case report: The study investigates a 36-year-old subject, hospitalized in the emergency room for traumatic shock after a traffic accident. He underwent surgery for left nephrectomy because of renal laceration, but died of these injuries: left temporo-parietal hemorrhage with subarachnoidal hemorrhage; bilateral hemothorax; fracture of the right clavicle; multiple rib fractures with bilateral pulmonary parenchymal contused injuries; fractures of the fifth dorsal vertebra without spinal cord injury; and, liver and intestinal lesions.

Results of Toxicological Investigations: Toxicological analysis performed on body fluids showed high levels of ethanol in bile, vitreous humor, and blood, as well as high levels of methadone. The values of ethyl alcohol in the blood were of 2.29g/l. Because of blood transfusion, the concentration of alcohol in vitreous humor had to be estimated. In effect, the vitreous humor levels should be in equilibrium with blood levels. The vitreous humor levels were the lesser affected of the two because the blood had been diluted by transfusion. Additionally, because the value of ethyl alcohol in the vitreous humor reaches a chemical equilibrium with a ratio of about 1:1 including the lymph and circulatory fluids, the vitreous humor levels were less affected. Many calculations were carried out to evaluate the value of ethyl alcohol in the blood at the time of the first transfusion. This result is shown through the application of appropriate correction factors that have considered the amount of blood transfused (1400ml), the weight of the subject, the metabolism of alcohol, the metabolic capacity medium, and the time elapsed from ingestion to accident. These calculations have determined the value of hypothetical blood alcohol at the time of the car crash before transfusion (1.28g/l).

Conclusions: In this case, it was possible to determine the concentration of blood alcohol levels over and above the cut-off. It has been concluded that the person was driving under the influence of alcohol. This investigation is essential for judicial purposes, in particular when it comes to an accident involving people who are the driver's responsibility. The study allows evaluation of a theme that has great social impact—it is very important to evaluate the conduct of the driver at the time of the incident.

Alcohol, Blood Transfusion, Toxicological Investigation

K24 Preclinical Investigation of CP47,497: A Widely Abused Synthetic Cannabinoid

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The goal of this presentation is to educate attendees about CP47,497 (2-[(1R,3S)-3-Hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol), a synthetic cannabinoid recently banned by the DEA. The pharmacology of this compound was first described in the scientific literature in 1982, but prior to its ban was being sold for consumption primarily through internet sources or head shops and was associated with a large spike in emergency department visits. CP47,497 activates the cannabinoid receptor 1 (CB_1R) and dose-dependently elicits cannabimimetic effects that are more potent than effects produced from Δ^9- tetrahydrocannabinol (THC). Importantly, these studies provide novel evidence using a whole animal model that the CB_1R antagonist rimonabant reverses the potent cannabimimetic effects of CP47,497

This presentation will impact the forensic science community by providing a direct comparison of synthetic cannabinoid and THC behavioral data in whole animal studies.

CP47,497 and other synthetic cannabinoid compounds were originally synthesized as tools to investigate the mechanism by which marijuana affects the brain as well as for the development of potential therapeutic agents to treat pain and other disorders. However, studies addressing the behavioral consequences of synthetic cannabinoids are scant. Synthetic cannabinoids pose an enhanced risk for abuse, toxicity, and addiction due their increased potency and efficacy over THC. The goal of the present study was to determine whether the pharmacological effects of CP47,497 are achieved in a dose-dependent and time-dependent manner. Since CP47,497 binds to CB_1 receptors and elicits THC-like effects, it was investigated whether rimonabant would attenuate its pharmacological actions *in vivo*.

All mice received intraperitoneal injections of CP47,497, THC, or vehicle. To test for cannabimimetic subjective effects, a tetrad model was utilized that consisted of four outcome measures: catalepsy; antinociception (tail flick latency); hypothermia; and, locomotor activity. Although many pharmacological agents can produce one or

a subset of these effects, drugs that activate CB_1 receptors produce measurable effects in all four parameters of the tetrad. Immediately following behavioral testing, mice were humanely euthanized and blood and tissue were harvested for CP47,497 quantification. Samples are currently being analyzed on an Applied Biosystems Liquid Chromatograph/Tandem Mass Spectrometer (LC/MS/MS) interface utilizing electrospray ionization and selective ion monitoring, using an acetonitrile liquid-liquid extraction procedure that the laboratory has previously developed validated methods for quantification of THC and other cannabinoids in blood and tissue.

In the cumulative dose-response experiment, mice were treated with THC (3, 10, 30, 100, and 200mg/kg), CP47, 497 (0.3, 1, 3, 10 and 30mg/kg), and vehicle control. Potency ratios for comparison of CP47,497 to THC were calculated including 95% confidence limits for each: catalepsy 7.49 (5.72 – 9.76), antinociception 9.11 (3.76 – 21.98), and hypothermia 7.68 (4.55 – 12.83), which clearly demonstrate CP47,497's enhanced potency and efficacy. In the final component of the tetrad, 30mg/kg CP47,497 produced a statistically significant increase in locomotor depressing effects versus control. Based on the data obtained from the dose-response study, 30mg/kg CP47,497 and 100mg/kg THC were used in subsequent antagonism studies. Both 30mg/kg CP47,497 and 100mg/kg THC produced statistically significant increases in catalepsy, hypothermia, antinociception, and a decrease in locomotor activity versus control. CP47,497 and THC-induced catalepsy and hypothermia were reversed by pretreatment with 3mg/kg rimonabant. Although 3mg/kg rimonabant antagonized the antinociceptive effects of 100mg/kg THC, 10mg/kg rimonabant was required to block the antinociceptive and locomotor depressing effects of 30mg/kg CP47,497.

This study's results provide the first *in vivo* evidence that the cannabimimetic effects of CP47,497 are CB_1 mediated as blockade of these effects is achieved with the CB_1 antagonist, rimonabant. Given that CP47,497 elicits dose-dependent cannabimimetic effects that are markedly (7 – 9 times) more potent than THC-containing substances, these data are consistent with the large number of abusers of this compound presenting with severe cannabis-related adverse effects that require emergency department interventions.

CP47,497, Spice, THC

K25 DART® AccuTOF™: A New Drug Screening Protocol for Biological Specimens

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After attending this presentation, attendees will learn how the DART® AccuTOF's™ technology can be utilized as a comprehensive screening technique of whole blood.

This presentation will impact the forensic science community by expanding the scope of analysis of whole blood drug screening to include targets not covered by traditional immunoassay techniques.

The field of forensic toxicology is never stagnant; preferences for a specific drug and/or drug combination fluctuate within the population. Meanwhile, scientists in the field are charged with providing timely, comprehensive, and accurate results while enduring dwindling personnel and financial resources, which often necessitates a limited scope covering only the staples. In order to comply with the duty of identifying both conventional and emerging drugs that are problematic in society, new methodology must be adopted in the screening of biological samples.

Current drug screening practices utilize both traditional immunoassay methodology and Gas Chromatography/Mass Spectrometry (GC/MS) technology. Immunoassays have been successful for the analyses of conventional drugs; however, immunoassays are costly, making scope expansion outside common drugs of abuse monetarily impractical. Additionally, immunoassays are limited for their ability to adapt quickly in the analysis of new and/or additional compounds due to kit production and/or validation. The GC/MS has less scope limitations when compared to the immunoassay but is a great deal more costly with respects to time. GC/MS technology requires tedious sample preparation prior to data collection and consumes multiple days of a scientist's time to complete the extraction, collection, and analysis of the data. Moreover, when used for drug screening purposes, any positive findings must then be confirmed by repetition. The Direct Analysis in Real Time (DART®) ionization source coupled to an AccuTOF™ mass spectrometer offers a solution to the restricted budget, available personnel, and drug screening limitations currently faced. Furthermore, DART® AccuTOF™ technology offers a second methodology for the screening of targets that have been traditionally identified and confirmed by repetitive GC/MS analyses. The robust, open air DART® ionization allows for comprehensive analysis by producing protonated molecular ions for all mode specific (positive mode) ionizable components of the specimen sampled via surface ionization, while the AccuTOF™ mass spectrometer allows for continuous data collection.[1,2] This hybrid instrumentation allows for the putative identification of both parent and metabolite compounds alike via a molecular formula database search with a total instrument analysis time of a couple of minutes per sample.

The application of the DART® AccuTOF™ technologies in the field of toxicology for the screening of whole blood, an exceedingly complex matrix, has realized the necessity for sample preparation prior to analysis.[3] To combat the complexity of the whole blood matrix, Disposal Pipette Extraction (DPX™) tips utilizing a cationic sorbent, featuring sulfonic acid groups, were employed for analysis of basic drugs spiked into porcine whole blood.[4] The amount of blood needed for the analysis was based upon the Limit Of Detection (LOD) study performed with neat standards. Porcine blood was screened for 35 different targets spanning a multitude of drug classes. Detected target coverage included basic and amphoteric compounds in the following classes: cathinones; sympathomimetic amines; select opiates; select benzodiazepines; dextromethorphan; carbamazepine; carisoprodol; select barbiturates; zolpidem; cocaine metabolite; citalopram; tapentadol; and, select tricyclic antidepressants. These detected targets were identified at therapeutic levels ranging from 10ng/mL to 400ng/mL.

Based on the experimental data collected, comprehensive screening can be accomplished with DART® AccuTOF™ technology. This study expanded the current drug screening scope of whole blood beyond the classical impairing drugs and even identified emerging select cathinones (bath salts). In conclusion, DART® AccuTOF™ technology has provided a promising solution to the current drug screening limitations encountered by forensic toxicologists.

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Whole Blood, DART® AccuTOF™, DPX tips

K26 More Bang for Your Buck—An Alternative Approach to Blood and Tissue Screening That Saves Time and Money

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After attending this presentation, attendees will have seen an alternative automated solid phase extraction technique to blood and tissue screening as compared to Liquid-Liquid Extraction (LLE) of basic drugs. Better recovery of designer drugs such as "bath salts" will also be shown.

This presentation will impact the forensic science community by demonstrating how more information can be obtained from the same sample volume, while saving time and money.

The Toxicology Laboratory at the Miami-Dade Medical Examiner Department recently changed the blood and tissue screening methodology from a multi-step LLE of basic drugs to a dual-Elution Solid Phase Extraction (SPE) of acidic/neutral and basic drugs. This was done to achieve a more cost-effective comprehensive blood drug screen, which utilizes smaller solvent volume and reduces sample preparation time.

The objective is to present data comparing the previously utilized LLE procedure to the newly implemented automated SPE method. Examples will include spiked controls, proficiency samples, and postmortem cases.

The LLE procedure (described by Forrester et. al in JAT) applies strictly to the extraction of basic drugs from 1mL of sample. The extract is then analyzed by dual column Gas Chromatography with Thermionic Sensitive Detection (GC/TSD).

The SPE method is a modified version of United Chemical Technologies Procedure Code: DRB200DAUZ120392 using UCT Clean Screen® cartridges and an automated Zymark Rapid Trace® system. The procedure uses 1mL sample volume yet yields two distinct fractions. The acidic/neutral extract is submitted for analysis by dual column Gas Chromatograph-Flame Ionization Detector (GC-FID), and the basic extract is analyzed by GC/TSD. GC-Ion Trap/MS is performed to confirm any positive findings.

The SPE method detected all 113 spiked control drugs and showed improved recovery for certain drugs, particularly the sympathomimetic amines and benzodiazepines. Co-elution of doxylamine and etomidate with caffeine was prevented since caffeine now elutes in the acidic/neutral extract.

The improved detection of ephedrine, in addition to the detection of acetaminophen in the acidic/neutral extract was noted in two separate proficiency samples in which these drugs were missed when screened using the former LLE method.

Screening of postmortem case samples utilizing the SPE method has led to detection of drugs in the acidic/neutral extract such as propofol, topiramate, levetiracetam, acetaminophen, and valproic acid which would have previously been missed. Newer drugs detected in the basic extracts include BZP, TFMPP, 5MeoDIPT, methylone, and MDPV, which could have been missed due to decreased recovery by LLE. In addition heroin, 6-MAM, morphine, and benzoylecgonine were detected in the initial GC/MS screening, as opposed to having to be specifically targeted in other confirmatory assays.

With the constant evolution of designer drugs, it is important for laboratories to respond and adapt accordingly, even though funding for consumables and staff may be limited. By adopting an SPE protocol, the laboratory is now equipped to screen for a variety of tryptamines, as well as the components of the ever-so-popular "bath salts." Additionally, the laboratory has become more efficient due to the reduction in solvent usage and sample preparation time. Other advantages include safety improvements and prevention of errors from multi-step procedures.

More information is obtained from the same sample volume via the dual elution which provides a much more comprehensive screen.

SPE, Comprehensive Screen, Basic Drugs

K27 Advanced Automated Library Searching for Compound Identification in Forensic Toxicology Samples

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After attending this presentation, attendees will learn about the different complimentary screening techniques that can be performed on a hybrid quadrupole linear ion trap to confidently identify targeted and unknown compounds. Attendees will learn about using acquired Tandem Mass Spectrometry (MS/MS) data to search against MS/MS libraries and utilizing a new advanced automated library searching with the capability to dynamically review the collected MS/MS information. Attendees will see that the software substantially improves the "data mining" process and provides an elegant solution to automated processing of the forensic toxicology screening data to confidently identify compounds.

This presentation will impact the forensic science community by demonstrating the advantages of the new automated library-searching approach in improving typical forensic toxicology screening workflows.

Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) utilization in forensic toxicology screening for drugs and drug metabolites has become increasingly popular due to the selectivity, sensitivity, and the speed of LC/MS/MS analysis. MS/MS confirmation with automated searching against available spectral libraries has proven to add a superior level of confidence to the compound identification. One of the key factors of the complete solution for forensic toxicology screening is automation of the library searching with the advanced capability to dynamically review the acquired data. Solutions need to be accurate and robust. The ability to search multiple libraries, create subsets of libraries, adjust and refine search parameters as well as re-search acquired data provides the user with the substantial flexibility. Setting mass tolerance, intensity thresholds, and searching multiple collision energies enhance the data under revision. The ability to search or re-search entire data files or a specific mass spectrum with different parameters can improve overall data quality and throughput. Additionally, reporting tools allow the information to be disseminated to the end user.

Forensic toxicology samples were analyzed using generic sample preparation procedures with two AB SCIEX LC/MS systems: a hybrid linear ion trap-triple quadrupole system and a hybrid quadrupole-time-of-flight instrument. The tandem mass spectrometric measurements were performed using the Collision Energy Spread (CES) feature which ensures the detection of the fragment ions generated in low-, medium-, and high-collision energy regimes. All the collected MS/MS spectra were searched against an AB SCIEX Forensic Drug Spectral Library comprised of over 1,250 compounds. The data processing was performed with the new AB SCIEX prototype library searching tool equipped with two library search algorithms. The accuracy, flexibility, speed, and robustness of the new library searching approach was successfully demonstrated in the processing of the data specifically acquired in different experimental set-ups. The ion trap screening data were collected in three screening workflows that consisted of several looped experiments as follows:

1. Multitargeted Screening:
    a. MRM detection of 300 analytes with the *Scheduled* MRM™ algorithm.
    b. Enhanced Product Ion (EPI) dependent scans set to automatically collect MS/MS fragmentation spectra for the targets detected in experiment 1.

2. General Unknown Screening:
    a. Enhanced Mass Spectrum (EMS) monitoring for the detection of the unknown analytes.
    b. EPI dependent scans set to automatically collect MS/MS fragmentation spectra for the unknowns detected in experiment 1.
3. Combined multitargeted and unknown screening:
    a. MRM detection of 300 analytes with the *Scheduled* MRM™ algorithm.
    b. EMS monitoring for the detection of the unknown analytes.
    c. EPI dependent scans set to automatically collect MS/MS fragmentation spectra for the targets identified in experiment 1 and unknowns identified in experiment 2.

The hybrid quadrupole-time-of-flight data collected using a TOF/MS survey scan with IDA-triggering of up to 20 product ion scans was also processed. In all specified cases, both targeted and unknown drugs and metabolites were identified in selected samples with a high level of confidence (based on the values of purity, fit, and reverse fit). Utilization of the new advanced automated library searching with the capability to dynamically review the collected MS/MS information has been demonstrated to substantially improve the "data mining" process and provide an elegant solution to automated processing of the forensic toxicology screening data.

LC/MS/MS, Library Searching, Hybrid Linear Ion Trap

K28 An Easy, Fast, and Reliable Workflow to Perform Real Forensic/Toxicological General Unknown Screening

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After attending this presentation, attendees will learn about a comparative screening workflow that allows the comparison between a sample and control in which significant differences in the sample are automatically extracted resulting in a reduction of several hundred peaks down to identifying only the significant components of the sample. Learning outcomes will include how high resolution accurate mass instrumentation can be successfully used to provide comprehensive and valuable information in identification of unknowns. Currently real General Unknown Screening suffers from complexity of biological matrices, which makes it almost impossible to identify relevant compounds in an easy and fast way. This presentation will present an easy-to-use generic workflow for General Unknown Screening. As an example, Tramadol in urine will be shown to be easily detected with only two injections in a Comparative Screening workflow using a hybrid quadrupole/time-of-flight instrument.

This presentation will impact the forensic science community by showing a fast, confident, and easy-to-use workflow to perform real non-targeted screening. The General Unknown Comparative Screening workflow provides basic sensitivity in Mass Spectrometry (MS) and Tandem Mass Spectrometry (MS/MS) modes for a clear identification of compounds, high resolution to overcome selectivity issues, and mass accuracy to capitalize from the provided resolution.

Method: ekspert™ ultraLC device was coupled to a fast-scanning high resolution MS system providing fast and sensitive MS/MS capabilities. Information-dependant acquisition with dynamic background subtraction and dynamic exclusion triggered 10 MS/MS experiments. The resulting total cycle time ensured that the compounds had more than 10 data points across extracted ion chromatograms (peak width 4 – 5 sec). Total LC runtime was 10 minutes using 6 min gradient (95% de-ionised water to 0% de-ionised water) with Phenomenex Kinetex 2.6µm C18 Column, 100 Å, 50 x 2.1mm column.

Results: The Comparative Screening workflow required two injections; a control injection was followed by the sample injection. The control was a urine sample of approximately comparable matrix to the sample without any drugs. Both data were loaded into PeakView™ software and automatically evaluated by an additional software add-on. All peaks overcoming a defined threshold were evaluated for retention time similarities in both sample and control. Significant differences in the sample due to, for example, absence or lower abundance of the same peak in control were automatically extracted and MS as well as MS/MS information was displayed (defined by second threshold). Thus, a reduction of several hundred peaks down to what is specific to the sample only were identified; Tramadol and its related major metabolites (demethylation). Sensitive MS/MS information can be used for confident identification by automatic searching MS/MS forensic library (1,250 entries). In case of missing conformity of a detected mass with any compound in any library, additional built-in software tools help to identify formulas by accurate mass, isotopic ratio, and sensitive MS/MS information. Finally, potential structures of calculated formula can be verified by fragment-predictive software tools.

Comparative General, LC/MS/MS, Accurate Mass

K29 Analysis of the Rate of Decay and Dispersion of Pentobarbital in Soil by Liquid Chromatography/Mass Spectrometry

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The goals of this presentation are to: (1) become familiar with the principles of extracting pharmaceuticals, specifically barbiturates, out of a complex matrix, particularly soil; (2) apply the use of this method to determine the initial concentration of the contaminated area and the focal point of contamination; and, (3) understand the significance of this method in order to determine the time of contamination based on the rate of decay and dispersion dynamics.

This presentation will impact the forensic science community by showing an effective method for extracting barbiturates out of complex matrices, specifically soil, is necessary for analytical analysis and has significant impact in the field of forensics and environmental science.

A method for detecting the barbiturate pentobarbital in soil previously developed was utilized to determine its application to the decay rate and dispersion rate of pentobarbital and similar barbituric acid derivatives in soil.

Pentobarbital is a pyrimidine derivative in a class of organic drugs called barbiturates. Several thousand derivatives of barbituric acid have been synthesized with far-reaching effects and flexible durations of action. Duration of action refers to the length of time the drug affects the target system, and in the case of humans or animals, it is the Central Nervous System (CNS). Pentobarbital is categorized as a fast-intermediate sedative-hypnotic drug. Barbiturates are highly stable organic compounds that are released into the environment via multiple pathways. Barbiturates have been extensively used throughout the United States. Euthanized animals are a growing contamination source in addition to the contribution of barbiturates from a wide array of pharmaceutical use, misuse, and abuse.

The method was developed to quantify the rate of decay of pentobarbital in contaminated soil. Pentobarbital in addition to other barbituric acid derivatives were extracted from separate soil samples, each separately spiked with the respective barbituric acid derivative. Clean-up procedures involved centrifugation, reverse-phase Solid Phase Extraction (SPE), microfiltration, and lastly, analysis by Liquid Chromatography/Mass Spectrometry (LC/MS). Concentration determination and recovery were determined utilizing a deuterated isotope method, Pentobarbital-D5, and an internal standard method. Satisfactory recoveries of the barbituric acid derivatives indicate this is an effective method for analysis and detection. Further, pre-

concentration via solid phase extraction allowed for 0.001mg of barbituric acid derivative per five grams of soil (200 parts per billion) to be detectable at limits of quantification using LC/MS. This method can be suitable for larger quantities of soil and applicable for a wide range of soil types.

The development of extraction methods for pharmaceuticals out of soil has multiple applications in the scientific community. Additionally, the application of this extraction method as to the determination of the source of contamination, date of contamination, and amount of contamination has significant impact in the field of forensics.

Barbiturate, Soil, Decay

K30 Method Development and Validation of Dimethylamylamine (DMAA, Methylhexanamine) by Gas Chromatography-Mass Spectrometry

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After attending this presentation, attendees will understand how a method was developed and validated for the detection of Dimethylamylamine (DMAA, methylhexanamine) in nutritional supplements and urine samples using Gas Chromatography/Mass Spectrometry (GC/MS).

This presentation will impact the forensic science community by providing a method for the detection of the emerging drug of abuse, and banned stimulant drug DMAA, and raise awareness of its use and presence in over-the-counter supplements and "legal highs."

Marketed as a nasal decongestant in the 1940s, DMAA, also known as methylhexanamine, currently has no recognized medical use. In 2006, the compound began to be added to nutritional supplements such as weight-loss preparations and pre-exercise pills. DMAA is a central nervous system stimulant which in excess produces effects similar to, but not as intense as, amphetamine. Combined with its availability and perceived low toxicity, DMAA is highly susceptible to abuse. DMAA use was recently associated with the death of two United States soldiers, was added to the World Anti-Doping Agency's Prohibited List, and is currently restricted in several countries.

As the compound is restricted in a number of countries worldwide, it is important to have an established, reliable method for its detection. Quantifying the concentration of DMAA in popular nutritional supplements will also benefit the forensic science community to the extent of determining just how potent and dangerous these supplements are to the public.

The objective of this research was to develop and validate a method for the detection of methylhexanamine in nutritional supplements and urine samples using GC/MS.

The analysis of DMAA is more challenging due to its two diastereomers and reactive primary amine group. After reconstitution studies with methanol, acetonitrile, isopropanol, ethyl acetate, and dichloromethane, DMAA was determined to be insufficiently stable for proper analysis on the GC/MS without derivatization. Following time and temperature studies, a successful derivatization method using 4-carbethoxyhexafluorobutyrl chloride (4-CB) that produced the two expected DMAA chromatographic peaks was developed. Extracts were derivatized by addition of 4-CB with ethyl acetate at 70°C for 20 min. Detection was performed by selected ion monitoring by GC/MS, using amphetamine-d5 as an internal standard.

A Liquid-Liquid Extraction (LLE) technique was used to isolate DMAA using concentrated ammonium hydroxide and chloroform/isopropanol/n-heptane (50:17:33) as the organic extraction solvent. The solvent was removed and evaporated at 33°C under a stream of nitrogen gas. Successful calibration curves have been established across the concentration range 1 – 20µg/mL. The curves generated acceptable r^2 values of 0.997 for DMAA peak 1 and 0.998 for DMAA peak 2. Successful calibration curves have also been established across the concentration range of 10 – 100ng/mL. These curves generated acceptable r^2 values of 0.994 and 0.993 for DMAA peak 1 and DMAA peak 2 respectively. In addition, the limit of detection and limit of quantitation were both preliminarily determined to be better than 10ng/mL, which is determined to be acceptable for both the analysis of solid dosage materials and expected concentrations in biological fluids.

The method is being applied to analysis of DMAA in nutritional supplements containing the drug and to biological samples. The presentation will also review the pharmacology of DMAA and reported adverse effects.

DMAA, Supplements, GC/MS

K31 Profiling of Inhalants and Common Blood Alcohol Interferences Using Headspace-GC/FID and Headspace-GC/MS

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WITHDRAWN

K32 A Proposed Means for the Detection and Quantification of Bath Salts From Blood

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After attending this presentation, attendees will understand the potential for Gas Chromatography/Flame Ionization Detection (GC/FID) use as a means of detecting and quantifying synthetic cathinones (bath salts). In addition, attendees will be aware of an assay that successfully extracts bath salts from blood samples. Finally, attendees will be aware of the stability of these extracts over a short period of time.

This presentation will impact the forensic science community by demonstrating a method for the extraction, detection, and quantification of a subset of novel drugs of abuse, specifically bath salts. As these drugs are quickly being banned at both the state and federal level, it is critical that laboratories develop appropriate assays in a timely fashion. Adoption of the method described in this study, rather than in-house method development, would allow laboratories to more quickly add bath salts to the drugs of abuse which they can report. In addition, the stability study performed on the extracts will provide valuable information for validation studies that must be performed in each laboratory.

Drug abusers often attempt to circumvent controlled substance legislation by manufacturing and using novel compounds. Recently, synthetic derivatives of the natural alkaloid cathinone, more commonly known as bath salts, have been used by drug abusers seeking legal alternatives to more common drugs of abuse such as amphetamines. Because these compounds are novel, few laboratories have validated methodologies for the extraction, detection, and quantification of these compounds. This study proposes a method for this purpose.

This study utilized a basic alkaline extraction procedure to extract methcathinone, mephedrone, pentedrone, 4-methylethcathinone (4-MEC), methylone, α-pyrrolidinopentiophenone (α-PVP), butylone, and

methylenedioxypyrovalerone (MDPV) from spiked blood samples. These extracts were then subjected to both GC/FID and Gas Chromatography/Mass Spectrometry (GC/MS). Retention times were noted for the samples on GC/FID and compared for possible co-elution. The compounds separated well with the exception of the co-elution of α-PVP and butylone on channel one. However, there was clear separation on the second channel between these two compounds, allowing all compounds to be combined into a single master mix for curve generation. GC/MS results were used to verify the identity of the compounds. Both GC/FID and GC/MS showed a good response from all compounds, demonstrating that the methods used on the instruments were appropriate for detection of bath salts. Calibrators were then created for each compound at 0.02mg/L, 0.05mg/L, 0.15mg/L, 0.50mg/L, and 1.00mg/L. The calibrators were run on the GC/FID and the area ratio compared to the internal standard alphaprodine was used to create standard curves that could be used to calculate the concentration of each of the compounds. These curves were linear over the 0.02mg/L – 1.00mg/L range using all five points. Each curve achieved a minimum R^2 value of 0.995.

Following the establishment of curves on the GC/FID, a stability study was performed on the extracts to determine the stability of each compound at each concentration over a period of one week. The extracts were run each day for a week and their concentrations were charted to determine any change over time. The overall trend indicates that the compounds begin to degrade at concentrations greater than or equal to 0.15mg/L after a 24-hr period.

Samples from cases in which bath salts were previously detected were then re-extracted to determine for which matrices the assay was suitable. Samples included antemortem and postmortem blood, urine, muscle tissue, and vitreous fluid, all of which are common sample types available in forensic analysis. The compounds of interest were both detected and quantified via the assay, demonstrating the suitability of these matrices.

Synthetic Cathinones, GC-FID, Quantification

K33 Capillary Electrophoresis and Capillary Electrochromatography Mass Spectrometry for Charged and Neutral Drug Detection

Hanzhuo Fu, BS, Florida International Univ, Dept of Chemistry, 11200 SW 8th St, Miami, FL 33199; and Bruce R. McCord, PhD, Dept of Chemistry, Florida International Univ, University Park, Miami, FL 33199*

WITHDRAWN

K34 Comprehensive Toxicological Examination in a Case Involving Alleged Use of Bath Salts: The Importance of a Negative Result

Barry K. Logan, PhD, and Sherri L. Kacinko, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to define the appropriate scope of testing for alleged “Bath Salts” intoxication cases, and appreciate that negative toxicology findings can only be properly interpreted when the scope of testing is understood.

This presentation will impact the forensic science community by providing an approach to rule out drug intoxication with emerging hallucinogenic drugs as part of a complex death investigation. It will also emphasize for the public and the press the importance of waiting for completion of toxicological testing before making assumptions about the role for intoxicants in criminal or death investigations.

In the assessment of crimes of extreme violence or where there is evidence of delusional or psychotic behavior, increasingly suspicion falls on the possible role of emerging designer stimulants and hallucinogens. Similar behaviors, however, can result from mental illness, schizophrenia, or even intoxication with traditional recreational drugs or drugs of abuse.

The importance of a structured escalating set of analytical protocols for these types of investigations cannot be overstated. Once routine toxicological analysis rules out traditional recreation drugs of abuse, the routine testing can begin. A recent case involving a violent assault provides a good example of how the application of routine tests can be supplemented by tests for additional drugs relevant to the fact pattern of the investigation

In this case, routine drug testing did not reveal the presence of any drug which could explain the extremely violent nature of the attack. Further testing was needed. Additional testing for the common “bath salts” compounds, MDPV, mephedrone and methylone, was performed. In addition a comprehensive screen for a wide variety of both traditional and emerging stimulant and hallucinatory drugs (67 in total) was performed using Liquid Chromatography-Time Of Flight-Mass Spectrometry (LC-TOF/MS). This included tryptamines, designer phenethylamines, other stimulants, cathinones, traditional stimulant drugs including cocaine and its metabolites and amphetamines, methoxy derivatives of phenethylamines, LSD, mescaline, psilocin, and bufotenin. Additional gas chromatography-mass spectrometric analysis was performed and compared to additional libraries containing over 10,000 known designer drugs and their analogs. The chromatographic data were further scrutinized for the presence of unidentified peaks; however, none were found.

Further testing for a comprehensive scope of emerging synthetic cannabinoid compounds was performed also. These drugs have been linked to adverse effects including paranoia, anxiety, psychosis, and violent assaults. A total of 20 synthetic cannabinoid drugs were tested for, including JWH-018, JWH-073, JWH-250, AM-2201, JWH-200, RCS-4, JWH-210, AM-694, JWH-122, JWH-081, JWH-019, RCS-8, JWH-203, JWH-022, JWH-018 5-chloropentyl, UR-144, XLR-11, AM-2233, AM-1248, and A-796,260. None were detected in the blood samples from the case.

Some of these emerging designer drugs, especially in the 2C series and derivatives of MDPV, as well as traditional hallucinogens like LSD and psilocin, are known to have limited stability in biological fluids, so appropriate collection and storage conditions are important in investigation of some of these more labile compounds. Consequently, their use cannot be ruled out even when toxicological tests are negative, and this places more of a burden on the results from a thorough investigation of the scene and history and circumstantial evidence of drug residue and paraphernalia when they exist.

In this case, comprehensive testing of available blood samples starting with routine analytical tests, and the addition of more specialized tests targeted to the compounds of concern, showed the presence of no drugs. No physical evidence from the scene or from the history revealed the presence of specific esoteric drugs that might have been ingested. The reasonable conclusion when the history, scene, autopsy, and toxicological evidence is considered in this case is that designer drug use was not a factor in this case.

Bath Salts, Designer Drugs, Drugs of Abuse

K35 Death by "Legal Psychedelic Piperidines and Phenethylamines": Postmortem Tissue Distribution of Desoxypipradrol (2-DPMP) and 4-Chloro-2,5-Dimethoxyamphetamine (DOC)

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After attending this presentation, attendees will have a better understanding of the piperidine and amphetamine class designer drugs desoxypipradrol (2-DPMP) and 4-chloro-2,5-dimethoxyamphetamine (DOC) and their concentrations in postmortem matrices.

This presentation will impact the forensic science community by informing forensic professionals on new abuse trends for amphetamines and designer drugs, particularly in our youth. It adds to the relatively sparsely published data concerning the potential toxicity of these stimulant drugs and provides a comprehensive approach to extraction, detection, and quantification of these substances.

These type of compounds have recently achieved "epidemic" status for abuse by young people. 2-DPMP exhibits a cocaine-like binding profile while DOC is a long-acting agonist of serotonin receptors; the fluoroamphetamines stimulate release and prevent reuptake of dopamine, serotonin, and norepinephrine.

Toxicities observed are similar to amphetamine toxicity: tachycardia, nausea, hypertension (vasoconstriction), insomnia, hyperthermia, mydriasis, panic attack, and seizures with the added predominant neuropsychiatric features of hallucinations, paranoia, and agitation.

A 30-year-old White male called a friend and advised him he had been snorting "DOC," 4-chloro-2,5-dimethoxyamphetamine, and was "tripping" and needed assistance. EMS and police were called and found the individual lying face down, breathing, but unresponsive, convulsing, extremely warm to the touch, and sweating heavily. He was conveyed to the hospital with an initial diagnosis of an acute drug overdose. The decedent had a history of selling, manufacturing, and using illegal drugs. All indications were that the overdose occurred within the last three hours. The individual died 42 hrs later at the hospital. A collection of narcotics and drug paraphernalia were confiscated from the residence as evidence and later submitted for analysis.

An autopsy was performed at the Cuyahoga County Medical Examiner's Office. Autopsy findings included dilated cardiomyopathy with a 460-gram heart, cerebral edema, and edematous lungs. Heart and femoral blood, vitreous humor, bile, liver, brain (medulla), and gastric were submitted for a comprehensive toxicology analysis.

The heart blood was positive for amphetamine 0.058mg/L, methamphetamine 0.170mg/L, fentanyl 2.0ng/mL; norfentanyl 0.44ng/mL, acetaminophen, atropine, caffeine, cotinine, lidocaine, and nicotine. The femoral blood was not sufficient in volume for analysis. No antemortem admission blood samples were available for subsequent analysis.

Because of the decedent's drug history, further testing was performed to determine the presence of other possible phenethylamine and amphetamine class drugs. Samples were extracted at a basic pH into ethyl acetate. 2-DPMP, DOC, and the fluoroamphetamines were separated and detected by an Agilent GC/MS-EI in full scan mode with a Restek-DB5 capillary column.

Further confirmatory testing was performed at AIT Laboratories, Indianapolis, IN, for the 2-DPMP, DOC, and fluoroamphetamines. Specimens were extracted at a basic pH into n-butyl chloride. Separation and detection was completed by a Waters Acquity UPLC coupled to a Waters LCT Premier XE TOF mass spectrometer as well as a Waters Acquity UPLC coupled to a Waters Tandem Quadrupole Detector (TQD). The analytical column for both analyses was a Waters BEH C18, 2.1 x 100mm, 1.7µm particle size.

The concentrations for the subsequent testing are as follows: desoxypipradrol (2-DPMP) concentrations (mg/L) were 0.283, heart blood; 0.236, vitreous humor; 1.98mg/kg, liver; 0.817mg/kg, brain (medulla); >1.0, bile, and negative, in the gastric.

4-chloro-2,5-dimethoxy amphetamine (DOC) concentrations (mg/L) were 0.466, heart blood; 0.380, vitreous humor; 1.40mg/kg, liver; 1.09mg/kg, brain (medulla); and 2.04 in the bile. Fluoroamphetamine and fluoromethamphetamine were qualitatively present in all the specimens.

2-DPMP and DOC was found to be distributed among multiple matrices with values ranging from 0.236 to >1.0mg/L for 2-DPMP and 0.380 to 2.04mg/L for DOC. Tissues responsible for detoxification/excretion had higher concentrations of the drugs. 2-DPMP, DOC, and the fluoroamphetamines were present in all tissues analyzed except gastric.

Drug chemistry results from submitted drug and drug paraphernalia exhibits were found to contain the following: 2-fluoromethamphetamine; alprazolam; 2-(1-pyrrolidinyl)-(4-methylphenyl)-1-propanone (MPPP); methamphetamine; dimethyltryptamine (DMT); psilocin; cannabis; fluoromethamphetamine; 4-chloro-2,5-dimethoxyamphetamine (DOC); phencyclidine; and, lysergic acid.

This case was consistent with the suspicion that this was an acute drug exposure. The cause of death was ruled toxic metabolic encephalopathy due to mixed drug intoxication. The manner of death was ruled as accidental.

Desoxypipradrol (2-DPMP), 2,5-dimethoxyamphetamine (DOC), Designer Drugs

K36 *In Vitro* Formation of Acetylmorphine From Morphine and Aspirin in Gastric Contents and Water

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After attending this presentation, attendees will understand that it is possible to form acetylmorphine *in vitro* by incubating human gastric contents or Deionized (DI) water with morphine and aspirin.

This presentation will impact the forensic science community by suggesting that detection of 6-acetylmorphine (6-AM) may no longer solely be an indicator of heroin use.

Forensic toxicologists across the world have considered detection of 6-AM to be definitive evidence of heroin use. 6-AM was detected in an 85-year-old female with a history of a witnessed arrest in bed at a nursing home. The decedent was under hospice care for failure to thrive, and had a history of multiple strokes, syncope, hyperkalemia, osteoporosis, anemia, and osteomyelitis. There was no history of illicit drug use and the decedent was prescribed morphine sulfate elixir (Roxinol®). Whether she was taking aspirin (acetylsalicylic acid) was not recorded and salicylates were not detected by colorimetry. The manner of death was natural and the cause of death was ruled as bronchopneumonia due to hypertensive atherosclerotic cardiovascular disease with remote myocardial and cerebral infarcts.

What about the 6-AM? Is it possible that an individual may be taking morphine (pain management) and aspirin (anticoagulant), and the aspirin may acetylate morphine to produce acetylmorphine? In the present study, the possibility of formation of acetylmorphine when morphine is mixed in solution with aspirin was investigated.

Two opioid negative, postmortem gastric specimens were selected for this study, along with morphine sulfate-Extended Release (ER) tablets (15mg) and coated-aspirin tablets (325mg). Morphine and aspirin tablets were placed into 50mL samples of the two separate gastric specimens, as well as deionized water. The three morphine/aspirin solutions were incubated at 37°C for increasing lengths of time. A separate experiment was run in gastric contents using 15mg morphine sulfate powder in lieu of morphine extended-release tablets. One milliliter aliquots were taken from all samples at 10 min intervals up to one hour, and then at 90 min, two hours, and ultimately 26 hrs. Aliquots were extracted using a previously published UCT solid phase opiate procedure, and analyzed by GC/MS in SIM mode.

Acetylmorphine was detected in all of the samples containing morphine and aspirin in combination. Levels of acetylmorphine were greater in gastric contents than in DI water during the same incubation period. After 120 min, the 6-AM concentrations for the samples containing aspirin and an ER tablet were 21ng/mL and 25ng/mL in the gastric solutions, compared to 7ng/mL in water. After 26 hrs at room temperature, the gastric concentrations were 124ng/mL and 121ng/mL, and in water 27ng/mL. The increase in concentration of acetylmorphine in gastric was linear (R^2 = 0.99 and 0.98), while formation in water was non-linear (R^2 = 0.63). The results for morphine sulfate powder were essentially identical to those observed for ER tablets. The initial pH of the two gastric samples were 4.74 and 5.27, respectively; following the addition of the morphine/aspirin tablets and two hours incubation, final pH values were 3.86 and 3.92. The final pH of the water solution was 2.88. This study demonstrates that it is possible to form acetylmorphine *in vitro* by combining morphine and aspirin tablets in both postmortem gastric contents and deionized water. The compound produced in this study was identified as 6-AM by GC/MS. Further investigation must be done to determine whether the compound is actually 6-acetylmorphine, 3-acetylmorphine, or a mixture of the two compounds.

Does acetylmorphine form *in vivo*? In addition to the case described above, 10,602 specimens were assayed for opioids by a pain management laboratory using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS). Three cases containing acetylmorphine were found to be inconsistent with heroin usage. A single specimen was listed as having a prescription for morphine and contained codeine, morphine, and 6-AM; the other two specimens contained 6-AM but not morphine or codeine. Although *in vitro* formation of acetylmorphine has been demonstrated, these data indicate that *in vivo* formation from the co-administration of aspirin and morphine is unlikely to occur. This may be attributed to inconsistencies in elimination half-lives; half-lives are 13 – 20 minutes and 1.3 – 6.7 hours for aspirin and morphine, respectively.

Morphine, Acetylsalicylic Acid, Acetylmorphine

K37 An Investigation of the Binding of Benzodiazepines to Human Serum Albumin and the Effect on Quantitation in Blood Samples

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After attending this presentation, attendees will understand how the binding of benzodiazepines to Human Serum Albumin (HSA) can affect the quantitation of benzodiazepines in blood samples. Attendees will also be made aware of how the varying binding affinities of different benzodiazepines for human serum albumin can affect quantitation within specific sample preparation methods.

This presentation will impact the forensic science community by providing further pharmacological/toxicological information on benzodiazepines, a class of drug that is commonly used therapeutically and is increasingly being abused in social settings.

Benzodiazepines are commonly prescribed central nervous system depressants which are found in a wide variety of different medications, from sedatives and hypnotics to amnesiatics and anticonvulsants. Benzodiazepines are increasingly being used as recreational drugs, often in combination with other drugs such as opiates and alcohol. HSA is the most abundant plasma protein in humans. Many drugs, including benzodiazepines, bind reversibly to albumin with albumin then acting as a carrier for the drug. This binding can increase the apparent solubility of the drug in the plasma and can influence the distribution, metabolism, and excretion of the drugs. Quenching of albumin fluorescence can be used to study the interactions of these drugs with albumin and characterize the binding affinities and other important binding characteristics. In a preliminary investigation, the binding affinities and other binding characteristics for alrazolam, bromazepam, diazepam, flunitrazepam, flurazepam, lorazepam, oxazepam, temazepam, and triazolam to HSA were tabulated. The binding constants of the nine benzodiazepines ranged from $1.14x10^2$M for diazepam, having the lowest binding affinity, to $8.05x10^6$M for flunitrazepam, with the highest binding affinity. The binding of these drugs to HSA and the binding affinity of each benzodiazepine derivative may affect the quantitation of these drugs in blood. In the current research, different preparation methods were utilized on samples spiked with known amounts of benzodiazepine. Quantitation was accomplished using an Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) method with Multiple Reaction Monitoring (MRM) which utilized a C18 column and isocratic elution with 0.1% formic acid in methanol (60%) and 0.1% formic acid (40%) at a total flow rate of 0.3500mL/min. The temperature range was 40°C – 95°C. Within a preparation method, the effect of differing binding affinities on the quantitation was studied. In a dilute and shoot method, flunitrazepam, a benzodiazepine which was shown to have a high binding affinity for albumin, showed a significant difference when quantitated in samples containing human serum albumin compared to samples without human serum albumin. Samples containing HSA had calculated concentrations that were 31% – 50% lower than samples without HSA. Diazepam, which was shown to have a lower binding affinity for albumin, also showed a significant difference when quantitated in samples containing HSA compared to those without. Samples containing HSA had a calculated concentration that was 40% – 60% lower than samples without HSA. Other preparation methods were used; a comparison of these results will also be presented.

Benzodiazepines, Human Serum Albumin, Quantitation

K38 Intraosseous Fluid as Alternative Biological Specimen in Postmortem Toxicology Case Evaluations

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After attending this presentation, attendees will understand the usefulness and value of Intraosseous Fluid (IOF) as a possible toxicologic specimen in postmortem cases of medicolegal interest.

This presentation will impact the forensic science community by providing valuable information on the collection of IOF during autopsy as well as its analysis by Enzyme Linked Immunosorbent Assay (ELISA) for several commonly encountered drugs in postmortem toxicologic evaluations.

In San Francisco, sudden, unexpected, or violent deaths are investigated by the Office of the Chief Medical Examiner. Autopsies are performed and biological specimens are collected for laboratory tests. Such specimens commonly include blood (central/cardiac and peripheral), urine, liver, and vitreous humor. Blood and vitreous humor are routinely screened for ethanol and related compounds by headspace gas chromatography equipped with flame ionization detection. Blood (central/cardiac) and urine specimens are further screened by ELISA for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, fentanyl, methadone, opiates, oxycodone, phencyclidine, and tricyclic antidepressants using commercially available ELISA kits and by Gas Chromatography/Mass Spectrometry (GC/MS) in full scan mode for over 100 common drugs and metabolites. Confirmations and quantitations are normally carried out in blood (peripheral) and urine as necessary to complete the toxicologic evaluations.

After evisceration, IOF specimens were collected from 30 decedents and from four different parts of the body (right tibia, left tibia, right humerus, and left humerus) together with other standard autopsy specimens. IOF specimens were collected using donated EZ-IO® intraosseous systems by Vidacare in 10mL syringes with continuous suction provided by holding back the commercially available plungers with mosquito forceps. IOF specimens were then transferred into clean gray top test tubes. All specimens were refrigerated until the time of analysis.

Blood (central/cardiac) and urine specimens were screened by ELISA per the Office's standard testing protocol. Additionally, IOF specimens were screened by ELISA using commercially available kits donated by Venture Labs, Inc. The kits used in the IOF experiments were designed and validated for blood analyses and the analysis took place using blood drug cut-offs. The ELISA drug screening results in IOF specimens for cocaine, amphetamine, methamphetamine, opiates, oxycodone, methadone, tricyclic antidepressants, fentanyl, and phencyclidine closely correlated with the ELISA drug screening results in blood. Correlation between blood ELISA and IOF ELISA results was 100% for cocaine, opiates, methadone, phencyclidine, and fentanyl, over 90% for tricyclic antidepressants (91%), and oxycodone (93%) but dropped to 89% for cannabinoids, 75% for methamphetamine, and 69% for amphetamine. Additionally, it appears that body origin of the IOF specimen may contribute to the correlation between IOF ELISA results and blood ELISA results since IOF from the left humerus and the left tibia showed slightly higher correlation to the blood ELISA results (93% and 93%, respectively) than those from the right humerus (91%) and the right tibia (86%).

Further studies are needed to fully investigate the potential of IOF in postmortem toxicology including quantitation of drugs in IOF and evaluation of this fluid's susceptibility, if any, to postmortem redistribution and interval, two issues that often arise when dealing with drug concentrations in postmortem blood specimens. This limited study suggests that IOF specimens appear to be relatively easy to collect at autopsy using commercially available collection devices, permit the collection of enough specimen volume for ELISA testing and appear to closely mimic blood ELISA drug screening results. For these reasons, intraosseous fluid should be considered as an alternative biological specimen by forensic pathologists, coroners, medical examiners, and forensic toxicologists for drug screening by ELISA in postmortem toxicology investigations.

Intraosseous Fluid, Toxicology, ELISA

K39 Quantitative Analysis of Endogenous Levels of Gamma-Hydroxybutyric Acid (GHB) in Hair Samples Using Different Extraction Techniques

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After attending this presentation, attendees will understand how a series of freeze/thaw cycles can be used to extract Gamma-Hydroxybutyric Acid (GHB) from human hair samples. Attendees will learn how freeze/thaw cycles can be a quick and reliable method for the analysis of hair samples from individuals suspected of illicit drug use.

This presentation will impact the forensic science community by providing a quick extraction method to use for hair analysis of GHB. It is thought that in the future this extraction method can be used to extract other drugs from human hair samples. This extraction method will help to decrease the analysis time of hair samples when they are encountered in the forensic science community.

GHB is a short chain fatty acid that was originally used for medicinal purposes such as the treatment of alcoholism and clinical depression. In 2005, GHB was approved under the trade name Xyrem® to treat narcolepsy and cataplexy. Despite its approved medicinal applications, GHB is primarily associated with being a recreational drug and drug of abuse. Within the forensic science community, GHB is most often observed in cases of drug-facilitated sexual assaults. Due to its synergistic effects with alcohol and quick onset of amnesia, GHB is an attractive drug of abuse for criminals attempting sexual assault.

The half-life of GHB in a healthy individual is only 20 – 53 min, which leaves a narrow and abrupt window for detection. Urinalysis, a conventional technique for detecting illicit drug use, is difficult to use because GHB is undetectable in urine within twelve hours after ingestion. This presents an additional obstacle for forensic scientists because many sexual assaults are not reported within this twelve-hour time window. Hair analysis of GHB may prove useful in the detection of drug-facilitated assaults involving GHB by allowing for a longer detection window. Most current hair extraction techniques are time-consuming and require a significant amount of sample preparation. Extraction using freeze/thaw cycles requires less time and less sample preparation. The freeze/thaw cycles consist of first washing the hair and then placing the hair into ethanol, which is used to extract the GHB from the hair. Once the hair is placed into the ethanol, the samples are then placed in liquid nitrogen until the ethanol is frozen (about 30 sec). The sample is then left to thaw, which usually takes about 2 min. This process is repeated for a total of five times. Once the sample is dried down, it is then derivitized and reconstituted in acetonitrile and then analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). Quantitation of GHB extracted from hair was accomplished using a pulsed-splitless GC/MS method, which had a pulse pressure of 30.0psi that was held for 1.75 min. This method was determined to be sensitive and robust for analysis of GHB.

The current research indicates that freeze/thaw cycles are comparable to other extraction techniques. The freeze/thaw extraction has worked on multiple types of hair and has allowed for efficient detection and quantitation of endogenous levels of GHB in human hair samples. This method has been shown to detect endogenous levels of GHB at 0.1ng/mg of hair. This method should also prove useful in the analysis of hair samples where illicit GHB use is suspected.

Endogenous GHB, Hair Analysis, Freeze Thaw

K40 Development and Validation of an LC/MS/MS Method for the Detection of the Metabolites of JWH-018 and JWH-073 in Human Urine

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After attending this presentation, attendees will learn the analytical method for the determination of major metabolites of JWH-018 and JWH-073 in human urine, and the quantification results of authentic urine samples.

This presentation will impact the forensic science community by presenting the fully validated analytical method for the detection of the main metabolites of new designer drugs, JWH-018 and JWH-073, in urine.

Due to their cannabis-like effect, synthetic cannabinoids have attracted much public attention since 2008. Thus, elucidation of the metabolic pattern as well as detection of the intake of these drugs has been of major concern. In the present study, a sensitive and reliable analytical method was established and validated for the simultaneous determination of the metabolites of JWH-018 and JWH-073 in human urine. For the routine screening in urine, (ω) and (ω-1)-hydroxyl, carboxyl, and hydroxyindole metabolites were selected as target drug metabolites. The samples were prepared by solid-phase extraction and analyzed using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). The LODs were 0.025ng/mL or 0.1ng/mL and the LOQs were 2.5ng/mL for all analytes. The results of the intra- and inter-day precision and accuracy were satisfactory: <10% for precision and within ±10% for accuracy at low (2.5ng/mL) and high (75ng/mL) concentrations. In this analytical method, no significant matrix effect was observed and high recoveries for all metabolites were achieved. The described method was applied to 52 authentic urine samples suspicious of JWH-018 or JWH-073 abuse and the quantification results among samples were compared. Twenty-one of the samples (40%) were found positive for at least one metabolite of JWH-018 or JWH-073. Carboxylated metabolite of JWH-073 was detected in all analyzed samples, which could be due to the metabolism of JWH-018 in humans. However, (ω) or (ω-1)-hydroxyl metabolite of JWH-073 was detected in only 12 samples. And only a small amount of these metabolites was detected compared with JWH-018 metabolites in most of the analyzed samples. In 14 samples of a total of 21 samples, (ω-1) hydroxyl metabolite of JWH-018 was the most abundant metabolites, with a mean concentration ranging from 2.9 to 671.2ng/mL; however, in the rest of the samples, the relative concentration of (ω-1) hydroxyl metabolite of JWH-018 was very low (<LOQ-23.4ng/mL) compared with that of the most abundant metabolite in the respective sample. It can be assumed that herbal mixtures used by the suspects contain JWH-073 as an impurity. 6-hydroxyindole metabolite of JWH-018 was detected in samples where (ω-1) hydroxyl metabolite of JWH-018 was the most abundant metabolite. Similarly, 6-hydroxyindole metabolite of JWH-073 was detected in only two samples which contain (ω-1) hydroxyl metabolite of JWH-073 with a concentration of more than LOQ. These results suggest that at least three metabolites including (ω) and (ω-1)-hydroxyl and carboxyl metabolites should be simultaneously monitored to prove intake of JWH-018 or JWH-073. The variation in the concentrations of detected metabolites could be due to the dosage of the drug and time intervals between the use of the drug and urine collection. However, the absence of detailed information such as dosage, content of synthetic cannabinoids in herbal mixture, and urine collection time makes it difficult to interpret the variation of concentrations between metabolites in the pharmacokinetic aspects. Thus, further study for the estimation of the profiles of metabolite concentrations after JWH-018 or JWH-073 intake versus time will be essential. The developed analytical method will be useful for confirmation and quantification of the metabolites of JWH-018 and JWH-073 in urine in the field of forensic toxicology.

JWH-018 & JWH-073, Metabolite, LC/MS/MS

K41 Carisoprodol and Meprobamate Incidence in DUID Cases in the City and County of San Francisco

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After attending this presentation, attendees will understand the reasons carisoprodol and meprobamate can impair one's ability to safely operate a vehicle, the incidence of these two drugs in DUID cases during a three-year period (January 1, 2009 to December 31, 2011), the blood concentrations measured in these drivers, and the identity of other drugs found in the blood of these drivers.

This presentation will impact the forensic science community by adding to the existing body of literature information regarding driving behavior, drivers' symptomology, blood concentrations, and other drugs found present in the blood of drivers driving under the influence of carisoprodol and meprobamate.

Carisoprodol and meprobamate are medications that are available only by prescription in the U.S. Carisoprodol is a muscle relaxant and meprobamate is a central nervous system depressant and a major metabolite of carisoprodol. Most patients are prescribed these medications for muscular pain management or anxiety, but abuse may develop due to the sedative-hypnotic effects these compounds produce.

In San Francisco, blood evidence is screened for ethanol and related compounds by Headspace/Gas Chromatography equipped with Flame Ionization Detection (HS/GC/FID). Blood is further screened by enzyme-linked immunosorbent assay for barbiturates, cannabinoids, cocaine, methadone, methamphetamine, opiates, and phencyclidine and by Gas Chromatography/Mass Spectrometry (GC/MS) in full scan mode for over 100 drugs and metabolites. Following a positive screening result for carisoprodol and/or meprobamate by GC/MS, a fresh aliquot of blood is extracted by liquid-liquid extraction and reanalyzed by GC/MS using quantitative calibrators freshly prepared in drug-free swine blood.

For the purposes of the present study, the in-house computerized database was interrogated, and 21 driving cases in which the laboratory had reported carisoprodol and/or meprobamate in blood specimens were identified during the three-year period of interest. Police reports were reviewed and information regarding the date/time of driving, the observed driving, and field sobriety test performance was collected. The 21 drivers with reportable carisoprodol/meprobamate in their blood comprised of 6 females and 15 males. The mean age of all drivers was 32 (range: 19 – 50). The mean age in females was 38 (range: 25 – 50) and 28 (range: 19 – 44) in males. Police reports indicated that drivers freely admitted to taking carisoprodol/meprobamate, did not exhibit breath odor of alcoholic beverage, displayed glossy, watery eyes, and slurred speech. Their demeanors were described as calm and relaxed. During the horizontal gaze nystagmus, clues observed included inability to follow the object, inability to keep head still and track only with eyes, lack of convergence, and lack of reaction to light. During the Romberg test, clues observed included eyelid fluttering, opening of the eyes during the test, and estimation of 38 – 95 sec for 30 sec. The clues observed during the "one leg stand test" included poor balance, inability to keep the foot off the ground, and raising arms up to six inches to maintain balance. Clues observed during the "finger count test" included

touching the finger pads instead of the tips and miscounting the steps and the number of steps performed.

Carisoprodol was reported in all 21 driving cases while meprobamate was reported in 20 of the 21 cases. The mean carisoprodol and meprobamate concentrations and associated ranges in all 21 drivers were 11mg/L (0.8 – 26mg/L) and 20.4mg/L (3.2 – 38mg/L), respectively. Carisoprodol and/or meprobamate were the only drugs detected in one-third of the cases included in this study (7 of 21 cases). In these seven cases, the mean carisoprodol and meprobamate concentrations were 12.3mg/L (7.3 – 16mg/L) and 30.4mg/L (19 – 36mg/L), respectively. In the remaining 14 cases where carisoprodol/meprobamate were not the sole compounds detected, drivers' blood specimens were found to contain on average two more psychoactive compounds including benzodiazepines (n=3), cannabinoids (n=3), oxycodone (n=3), ethanol (n=2), methadone (n=2), hydrocodone (n=2), cocaine/benzoylecgonine (n=2), methamphetamine (n=1), MDMA (n=1), citalopram (n=1), and tramadol (n=1).

Carisoprodol/meprobamate occurrence in driving under the influence cases in the City and County of San Francisco is a significant and on-going challenge. Often these drugs are present with other psychoactive compounds in drivers, making it difficult to assign specific signs and symptoms to them. Carisoprodol/meprobamate were the only drugs found in the blood of drivers in the minority of these 21 cases whereas in most of the cases, they were present together with several other compounds, primarily benzodiazepines, cannabinoids, and oxycodone.

Carisoprodol, Meprobamate, DUID

K42 Cannabinoids in 113 Driving Under the Influence of Drugs (DUID) Forensic Toxicology Cases

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After attending this presentation, attendees will understand the ranges of concentrations at which cannabinoids are often detected in DUID cases.

This presentation will impact the forensic science community by providing valuable information on cannabinoid incidence among drivers and by offering blood reference concentrations of common cannabinoids in DUID case investigations.

The Forensic Laboratory Division of the Office of the Chief Medical Examiner performs DUID and other human performance forensic toxicology cases investigations on behalf of 14 law enforcement agencies routinely operating within the City and County of San Francisco. Specifically for cannabinoids, commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits by Venture Labs, Inc. are employed to screen blood and urine. The ELISA cannabinoid cutoffs used for blood and urine are 5 ng/mL and 50ng/mL, respectively. Following a positive ELISA, confirmation and/or quantitation takes place on a new specimen aliquot by Gas Chromatography/Mass Spectrometry (GC/MS) with a limit of quantitation of 1ng/mL for Δ^9-tetrahydrocannabinol (THC) and 5ng/mL for 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) for both blood and urine. The deuterated compounds THC-d_3 and THCCOOH-d_3 are used as internal standards. For THC-d_3, the target (underlined) and qualifier ions are 389 and 374. For THCCOOH-d_3, the target (underlined) and two qualifier ions are 374, 476, and 491. For THC, the target (underlined) and qualifier ions are 386 and 371. For THC-COOH, the target (underlined) and two qualifier ions are 371, 473, and 488. Finally, for THC-OH, the target (underlined) and two qualifier ions are 371, 459, and 474.

Between July 1, 2010, and June 30, 2011, the Division performed toxicologic evaluations in 919 DUID cases including the cannabinoid protocol described above. Cannabinoids were consequently reported in 113 of the 919 cases (12.3%). Five of these cases involved urinalysis and 108 involved blood analysis.

Drivers averaged 29.7 years of age (range: 14 – 68 years) and were predominantly male (n=100; 88%) which represents a higher proportion of male drivers as compared to the overall sex distribution of drivers whose toxicology was performed by this Division during the same time period (750 males, 82%; 169 females, 18%).

Among all 108 blood DUID cases, mean concentrations and associated ranges in ng/mL for THC, THC-OH, and THC-COOH were 5 (1 – 33), 9 (5 – 14) and 52 (5 – 320), respectively.

In 64 of the 108 cases involving blood evidence, ethanol was also found at a mean concentration of 0.13% (w/v) which ranged from 0.01 – 0.38% (w/v). In four of these 64 ethanol-cannabis cases, additional drugs were also detected (diazepam/nordiazepam in two cases, alprazolam in one case, methadone in one case, and MDMA/MDA in one case). In the 64 cases where ethanol was reported in addition to cannabinoids, mean concentrations and associated ranges in ng/mL for THC, THC-OH, and THC-COOH were 4 (1 – 23), 9 (8 – 12), and 44 (5 – 150), respectively.

Cannabinoids were reported in combination with drugs other than ethanol in 15 cases. In those 15 cases, drugs found in addition to cannabinoids were codeine (four cases), cocaine/benzoylecgonine (three cases), methamphetamine/amphetamine (three cases), MDMA (three cases), oxycodone (two cases), alprazolam (two cases), methadone (one case), and hydrocodone (one case). In these 15 cases where cannabinoids were found in combination with drugs other than alcohol, mean concentrations and associated ranges in ng/mL for THC, THC-OH, and THC-COOH were 7 (1 – 33), 8 (7 – 10), and 65 (13 – 290), respectively.

Cannabinoids were the only compounds reported in 29 of the 108 blood DUID cases. In these 29 cases, mean concentrations and associated ranges in ng/mL for THC, THC-OH, and THC-COOH were 7 (1 – 26), 8 (5 – 14), and 62 (6 – 320), respectively.

In the five cases involving urinalysis, THC-COOH was the only cannabinoid confirmed present. Only in one of the five urinalysis cases, cannabinoids were the only compounds reported. Cocaine/benzoylecgonine, cocaethylene, and codeine were each confirmed present in two urinalysis cases, whereas morphine, phencyclidine, carisoprodol, meprobamate, promethazine, norpromethazine, and levamisole were each confirmed present in one urinalysis case. Per professional guidelines, drug concentrations are not measured/reported in urine specimens.

This study offers a significant insight into the blood cannabinoid concentrations of drivers involved in DUID investigations in San Francisco. The reported mean concentrations suggest that drivers who concurrently consume ethanol with cannabis have on average lower THC blood concentrations than drivers who use cannabis by itself or with drugs other than ethanol and one may infer these drivers may be changing their cannabis use patterns (i.e., consuming lower cannabis doses and/or extending the waiting times before drinking) when they combine cannabis with alcohol. This type of epidemiological data provides reference concentrations for forensic toxicologists, law enforcement agents, and attorneys who are required to evaluate cannabinoid concentrations in human performance toxicologic specimens when involved in DUID investigations.

Cannabinoids, Toxicology, DUID

K43 Toxicology Result of Drivers of Fatal Motor Vehicle Accidents in Harris County, Texas, in 2011

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After attending this presentation, attendees will have relevant information about the involvement of illicit, prescription, and over-the-counter drugs in causing the majority of fatal traffic accidents.

Attendees will learn the importance of extensive standardized testing of biological specimens for different classes of drugs in addition to alcohol.

This presentation will impact the forensic science community by raising awareness on the prevalence of fatal motor vehicle accidents caused by drugs alone with no alcohol involved. The involvement of drugs in fatal traffic accidents other than alcohol have not been adequately explored, partly due to lack of information in police training and most of the crime laboratories are neither mandated nor equipped to perform confirmation and quantitation of illicit, prescription, and over-the-counter drugs in biological specimens, especially blood. The other reason is the high cost of drug confirmation in biological specimens and the possibility of obtaining convictions based on only blood alcohol levels.

Driving under the influence of alcohol and drugs has been the main cause of fatal and non-fatal accidents for drivers and other occupants in the car, as well as pedestrians. In 2011, the medical examiner of the Harris County Institute of Forensic Sciences performed autopsies of 214 victims of fatal car crashes. All drivers of fatal motor vehicle accident cases were subject to alcohol screening and confirmation, nine-panel Enzyme-Linked Immuno-Sorbent Assay (ELISA) screens for drug of abuse, standard basic drug screen for prescription and over-the-counter drugs using Gas Chromatography/Mass Spectrometry (GC/MS), screens for 11 synthetic marijuana (Spice/k2) drugs, 8 methylcathinones (bath salt) drugs, and three hypnotic Z-drugs using Time-Of-Flight/Liquid Chromatography/Mass Spectrometry (TOF/LC/MS). All identified drugs by screening methods were subject to confirmation and quantitation by GC/MS, GC/MS/MS, and LC/MS/MS instruments. Out of 214 victims, 134 (63%) had ethanol and other drugs in their system. Out of 134 cases, 86 cases were positive for ethanol with quantitation result greater than 0.08gm/dl in 77 (89%) of cases and less than 0.08gm/dl in 9 (10.4 %) of cases. Out of 134 cases, 46 (34%) were positive for illicit, prescription, and over-the-counter drugs with no ethanol. Next to ethanol, the most common drug identified was marijuana in 30 (22%) of the cases with and without ethanol present. Ten (17%) of the cases had alprazolam, 9 (6.7%) cases had cocaine, 7 (8.9%) cases had hydrocodone, and 5 (3.7%) cases had PCP. The prevalence of alcohol and drugs among the deceased drivers' cases indicate alcohol and marijuana being the most common findings followed by benzodiazepines, opiates, cocaine, PCP, muscle relaxants, and other prescription drugs. Out of 134 drug-positive cases, 100 (74%) are male and 26% are female drivers, 63 (47%) are White, 48 (35%) are Hispanic, 27(20%) are Black, 3 (2%) are Asian and 2 (1.4%) are unknown race. The most common age group is in the range of 21 – 30 (35%), followed by 31 – 40 (20%), 41 – 50 (15%), 51 – 60 (17%), >60 (8.2%), <21 (11%). White male drivers, 21 – 30 years of age, are the most identified victims of fatal alcohol and drug related accidents.

The importance of screening and confirmation of illicit, prescription, and over-the-counter drugs in the biological specimen of motor vehicle accident victims is discussed.

Drugs, Driver, Fatality

K44 Using Pharmacology to Screen Your DWI-Drug Cases

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After attending this presentation, attendees will be able to describe the pharmacologic criteria necessary for a prosecutor to meet the burden of proof to establish DWI-Drugs and apply these criteria to the findings of the presence of a drug in the urine.

This presentation will impact the forensic science community by presenting pharmacologic criteria which will help prosecutors select meritorious cases and defer going forward on cases when the pharmacology clearly will not support guilt beyond a reasonable doubt. An awareness of these criteria should minimize the prosecution of unwinnable cases, save valuable court time, improve the quality of justice in the courts, and protect innocent citizens from unnecessary mental anguish and monetary expense. A prototypical case will be discussed.

In order to successfully prosecute a DWI-Drugs case, the State must show that the defendant driver was impaired, and that the impairment was proximately caused by a previously ingested drug. The drug could be an illicit drug, a prescription drug, an over-the-counter drug, or an inhalant of some type. An admission by the driver that he/she took a drug earlier, or the positive results of a urine drug test, only indicate prior exposure, but do not demonstrate that the previously ingested drug proximately caused impairment at the time of the police stop. Evidence of impairment is best established by observations of erratic behavior by fact witnesses, and testing of balance, coordination, and cognitive function by professionals qualified to administer such tests.

Example of an Unwinnable DWI-Drug Case: In a Commonwealth of Massachusetts case (Com), a young woman, E.D., was stopped for crossing a road line. Her urine screen was positive for a small amount of butalbital, a barbiturate found in common prescription anti-migraine medications.

Many drugs remain in the body long after the time interval of their pharmacologic activity has elapsed (biosphere). Thus, determining the presence of a drug in a biological fluid is not a sufficient basis to opine that an individual is impaired. Instead, one must determine the biosphere of that drug, that is, the duration of time the drug exerts its pharmacologic effect on the individual, rather than its residence time in the body. Moreover, only certain drugs show a good correlation between the magnitude of their blood level and the extent of the effect they produce on the individual. Other drugs, called hit-and-run drugs, produce an effect long after they have left the body and are no longer detectable in blood or urine. A good example of such a drug would be the glucocorticoid, prednisone, which produces major changes in the hypothalamic-pituitary-adrenal axis, which may persist for weeks or months.

The half-life of butalbital is 1.5 – 3.5 days. Based on generally accepted pharmacokinetic principles, it takes 6 – 10 half-lives to rid the body of a drug. This means that E.D. could have taken the drug more than a month earlier and still had a positive urine test on the day she was stopped, even though the pharmacologic effects on migraine relief and possible impairing effects last only a few hours, and tolerance to barbiturates is known to occur after repeated use.

The COM planned to have a Drug Recognition Expert (DRE) testify at trial that the defendant had been impaired, an opinion that could not be proven beyond a reasonable doubt, and one to which the DRE was not percipient, as she had never met or assessed the defendant. A DRE is basically a fact witness, not an expert, and according to *U.S. vs. Horn* (Motion Hearing, 2001) testifies under FRE 701 not FRE 702, and cannot offer opinion testimony on scientific or technical issues. However, on the day of the *Daubert* hearing to strike the DRE, the COM reconsidered its position, and agreed to let the defendant off with probation.

In this case, a simple pharmacokinetic analysis would have indicated the very low likelihood that the prosecution could have proved its case beyond a reasonable doubt, or that a jury would have convicted. The defendant was subjected to unnecessary anxiety and considerable expense, without an adequate basis for prosecution of this case.

Pharmacokinetics, Lay Witnesses, Expert Witnesses

K45 Cannabinoids in Exhaled Breath Following Controlled Administration of Cannabis

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The goal of this presentation is to offer a greater understanding of the applicability and relevance of measuring cannabinoids in breath following controlled smoked cannabis. The hypothesis is that the detection window of cannabinoids in breath will be several hours. This would be an appropriate timeframe for detecting driving under the influence of drugs, as this is similar to the window of acute drug impairment.

This presentation will impact the forensic science community by supporting the application of cannabinoid breath testing, particularly in the drugged driving field.

Methods: Breath specimens were collected using SensAbues (Huddinge) devices prior to and following *ad libitum* smoking of a single 6.8% Δ^9-tetrahydrocannabinol (THC) cigarette for 10 min in occasional and chronic cannabis users. This study was Institutional Review Board- (IRB) approved and written informed consent was obtained from participants. Breath specimens were collected during a 3 min period at -18, -1, 0.5, 1, 2, 3, 4, 5, 6, 8, 10.5, 13.5, and 21 hr post-smoking. Sample preparation involved a 20 min methanolic extraction, followed by solid phase extraction on polymeric SSTHC columns (UCT). THC, 11-nor-9-carboxy-THC (THCCOOH), and cannabinol (CBN) were quantified by Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) similar to a method proposed by Beck *et al*. with minor modifications.[1] Limits of Quantification (LOQs) were 50pg/pad for THC and CBN and 100pg/pad for THCCOOH. Linearity extended to 10,000pg/pad for all analytes. Extraction efficiencies for THC, THCCOOH and CBN were 33.8 34.6%, 58.1-63.0%, and 67.1-73.5% across the linear range. Matrix effects ranged from -34.6 to 12.3%. Extraction efficiencies and matrix effects were similar for matched deuterated internal standards. To date, breath specimens from one occasional and five chronic smokers were tested.

Results: Following controlled administration of THC, breath pads were positive only for THC; CBN and THCCOOH were not detected in any specimen at the method's LOQs. Breath pads were positive only at the 0.5, 1, and 2 hr post-smoking collections. Following cannabis smoking, dry mouth frequently occurred, making the collection of oral fluid difficult. As oral fluid collections occurred just prior to breath collection, breath collection times were delayed early in the time line due to prolonged oral fluid collection times. Mean (range) times for breath collection were 0.9 (0.78-0.98), 1.4 (1.30-1.45), and 2.4 hr (2.25-2.58) post-smoking. All but one participant had detectable THC in breath at 0.9 hr with a median (range, n) THC breath concentration of 147.0pg/pad (117-409, n=5). At 1.4 hr post-smoking, all but one participant had THC-positive breath with a median concentration of 122.2pg/pad (71.4-209, n=5). Only three participants still had detectable THC in breath at 2.4 hr post-smoking with a median concentration of 67.6pg/pad (54.0-86.3, n=3). For two participants, only one breath collection was positive for THC: 109pg/pad at 1.37 hr and 118pg/pad at 0.9 hr. Participants with multiple positive specimens (n=4) showed decreasing THC breath concentrations over time. In all participants, once breath specimens were negative for THC, they remained negative for the duration of the study.

Conclusions: The cannabinoid detection window in breath was short, ranging from 0.9 – 2.4 hr after cannabis smoking. Only parent THC was present; CBN and THCCOOH were not detected. Breath is an alternative matrix to oral fluid for a short cannabis detection window. Future research should determine if THC is present in breath during sustained cannabis abstinence in chronic daily cannabis smokers. During prolonged abstinence after chronic daily cannabis smoking, large THC body stores were slowly eliminated in the blood, plasma, urine, oral fluid, and sweat. The low THC concentrations in breath after cannabis smoking suggest that prolonged excretion will not occur. These data support the suitability of cannabinoid breath testing in the field of forensic science, particularly in the drugged driving field.

Reference:

1. Beck, O, Sandqvist, S, Dubbelboer, I and Franck, J. (2011) "Detection of Δ9-Tetrahydrocannabinol in Exhaled Breath Collected from Cannabis Users." J Anal Tox (35): 541-544.

Breath, Cannabinoids, THC

K46 Determination of Synthetic Cannabinoids in Whole Blood From Recreational Users

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After attending this presentation, attendees will understand how synthetic cannabinoids may be analyzed and will have an insight in the common findings in recreational users as well as the challenges these compounds present to the forensic toxicologist. This paper presents a Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) method for synthetic cannabinoids in whole blood and toxicological findings from recreational users.

This presentation will impact the forensic science community by adding findings for substances that have paucity in data.

Analysis was performed on a Waters ACQUITY ultra performance LC connected to an API 4000 triple-quadrupole instrument equipped with an electrospray interface. The following compounds were included in the method: JWH-007, JWH-015, JWH-018, JWH-019, JWH-020, JWH-073, JWH-073-methyl, JWH-081, JWH-098, JWH-122, JWH-147, JWH-200, JWH-203, JWH-210, JWH-250, JWH-251, JWH-398, AM-694, AM-2201, AM-1241, RCS-4, RCS-8, and WIN 55,212-2. JWH-018-d_{11} was used as internal standard. The analytes were chosen because they either were scheduled by the Swedish government or because they were reported as possible drugs of abuse.

To 1g whole blood was added 0.5mL TRIS-buffer (0.5M, pH=8.5) and 3mL of tert-butylmethylether:chlorobutane (50:50). The organic phase was transferred to new tubes, evaporated to dryness, and reconstituted in 100µL of 10mM ammonium formate and acetonitrile (50:50). 5µL was injected into the LC/MS/MS. Chromatography was performed using an ACQUITY high-strength silica T3 column (1.8µm, 50 x 2.1mm, Waters) and operated at 0.6mL/min with a total run time of 6 minutes. Mobile phase A consisted of 0.05% formic acid in 10mM ammonium formate and phase B was 0.05% formic acid in acetonitrile. The chromatographic system was run in a linear gradient from 35% to 70% phase B.

Method validation included selectivity and matrix effect studies, investigations of calibration models, accuracy, within and between day precision, and dilution integrity. The method was applied to 1,609 authentic cases where the police had requested synthetic cannabinoids.

The method validation experiments showed overall good results for the 23 analytes. Matrix effects were seen especially for the late eluting compounds and an interference for the transitions of RCS-8 appeared in most chromatograms close to the retention time of RCS-8. All analytes were best fitted to quadratic calibration curves between 0.05ng/g to 5.0ng/g.

The overall positive rate was approximately 30% with AM-2201 as the most common finding. The positive findings changed over time, sometimes so that substances that were scheduled decreased to be

replaced by new unscheduled analogs. Eleven of the analytes were found in one or more cases.

Analyte	*N*	Median (ng/g)	Mean (ng/g)	Range (ng/g)
JWH-018	80	0.10	0.62	0.05 – 9.7
JWH-019	7	0.65	0.44	0.07 – 0.78
JWH-081	8	0.20	0.24	0.06 – 0.65
JWH-122	51	0.42	3.6	0.05 – 88
JWH-203	3	13	27	0.06 – 68
JWH-210	31	0.75	1.6	0.07 – 11
JWH-250	3	0.48	0.45	0.14 – 0.73
AM-694	10	0.12	0.43	0.05 – 1.5
AM-2201	290	0.34	1.0	0.05 – 29
RCS-4	5	0.23	0.80	0.06 – 3.4

Concentrations were typically in the subnanogram range, but some cases had very high concentrations (see table). It has been reported that synthetic cannabinoids, in comparison to cannabis, seem to be more dangerous and potent, causing several unwanted symptoms in the users. In the material, case histories were not received in more than a few cases where the subjects had suffered from severe side effects and been brought to hospital. These subjects presented with unconsciousness, vomiting, incontinence, and hallucinations and relatively high concentrations of JWH-018, JWH-203, or JWH-210, sometimes in combination with another synthetic cannabinoid. This study concludes synthetic cannabinoids appear in very low concentrations and the changing panorama of substances requires a flexible approach to the analytical methodology.

Cannabinoids, LC/MS/MS, Recreational Users

K47 Driving Under the Influence of Alprazolam

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After attending this presentation, attendees will have a better understanding of the prevalence of alprazolam in DUID cases and the lack of correlation between blood alprazolam concentrations and impairment.

This presentation will impact the forensic science community by adding to the body of knowledge regarding alprazolam concentrations found in Driving Under the Influence of Drugs (DUID) cases, observed impairment, and comparison to the indicators for central nervous system depressants in the Drug Recognition Expert (DRE) matrix.

Alprazolam, a high-potency benzodiazepine, is FDA approved for the treatment of anxiety and panic disorder and is one of the most prescribed medications and abused drugs worldwide. Sedation and impairment of cognitive function and psychomotor performance are some of the main problems associated with the use of benzodiazepines as anxiolytics. Patients using alprazolam commonly report adverse events, such as drowsiness, dizziness, and reduced alertness, especially in the first month of use. Alprazolam is commonly used with alcohol and other recreational drugs, presumably to achieve increased intoxication and manage undesirable drug withdrawal symptoms such as the downside or dysphoric phase of stimulant use and to alleviate the panic and paranoia caused from using high potency cannabis. The pharmacology of alprazolam will be reviewed as an attempt to understand why Alprazolam is such a popularly abused drug in suspected intoxicated driver cases across the United States.

Between 2007 and 2012, 28% of all blood DUI drug cases submitted to the Palm Beach County Sheriff's Office Crime Laboratory contained alprazolam, making this the most commonly detected drug. Of the 205 cases analyzed during this period containing alprazolam, only nine contained alprazolam alone (4.4%). The nine subjects were composed of six males and three females with a mean age of 45 years (range 17 – 72). Seven out of nine involved traffic crashes. Alprazolam concentrations ranged from 22 to 437ng/mL.

During the first six months of 2012, 13% of all DRE cases submitted to the Washington State Patrol Toxicology Laboratory contained alprazolam. Of the 64 DRE cases analyzed during this period containing alprazolam, only seven contained alprazolam alone (10.3%). The seven subjects were composed of four males and three females with a mean age of 38 years (range 24 – 57). Alprazolam concentrations ranged from 10 to 210ng/mL.

Summary data from all sixteen alprazolam-only cases followed by detailed information for four cases from Palm Beach County and seven Washington State cases will be presented. Detailed information will include testing protocol, analytical results, and case synopsis including observed impairment and clinical indicators of drug use. The indicators for central nervous system depressants in the DRE matrix will be compared and contrasted with these case investigations.

Alprazolam, DUID, DRE

K48 Prevalence of Tetrahydrocannabinol in Oral Fluid Collected From Drivers in California

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After attending this presentation, attendees will be aware of the prevalence of marijuana in the oral fluid of California drivers. Attendees will appreciate the utility of oral fluid in traffic safety situations.

This presentation will impact the forensic science community by showing that oral fluid is a viable alternative to blood for the analysis of drugs in specimens taken from drivers.

Recent roadside surveys conducted in the United States have determined that cannabis is the most prevalent drug detected in drivers randomly stopped and voluntarily tested. The 2007 National Roadside Survey reported that 7.7% of nighttime weekend drivers tested positively for tetrahydrocannabinol (THC) in their oral fluid, indicating recent ingestion of cannabis.

This presentation will focus on data collected from drivers in various locations in California during 2010 and again in 2012.

In 2010, researchers attempted to recruit 1,784 drivers who were stopped at random during nighttime hours on Friday and Saturday evenings. Due to age or type of vehicle, 282 were not selected. Of the 1,502 eligible drivers, 297 either refused to be part of the survey, or only completed part of the process. In total, oral fluid was collected voluntarily from 900 drivers. Subjects were predominantly male (63.2%), White (60.5%), and had a median age of 29. A total of 14.4% of drivers tested positively for illegal drugs with 8.5% testing positively for THC. Only 1.3% of the drivers were positive for both THC and alcohol, a combination known to significantly increase the odds of traffic accidents. Compared to the 2007 National Survey, the percentage of marijuana positives in California drivers had increased overall (from 4.9% to 7.8%) and in three of the four comparable jurisdictions; only one showed a lower percentage of positive drivers in 2010. In 2010, the prevalence of cannabis varied throughout the state with Fresno showing the lowest prevalence of 4.3% and Eureka, Humboldt County, having the highest prevalence of drivers positive for marijuana, 18.3%. The concentration range for THC in oral fluid was 2 – 1284ng/mL; mean 199ng/mL; median 30ng/mL. Part of the

impetus for the 2010 study was the potential for decriminalization of marijuana in California Proposition 19—also known as the Regulate, Control, and Tax Cannabis Act of 2010—which was on the ballot in November 2010. If it had been approved, the proposition would have legalized various marijuana-related activities in California (although not under federal law), allowing local governments to regulate these activities, permitting local governments to impose and collect marijuana-related fees and taxes, and authorizing various criminal and civil penalties. The ballot initiative was defeated 53.5% to 46.5%. However, medical marijuana is legal in California (Proposition 215, 1996) and as part of the survey, drivers were asked if they held a permit for its medical use. While only 36 drivers admitted to having permits for medical marijuana, 38.9% of them tested positively for THC, compared to 7.5% of those without permits. When controlled for driver age, race, and including jurisdiction as a random variable, drivers holding permits for medical marijuana were significantly more likely to test positively for THC than nonpermit holders.

In 2012, the California Study was repeated with data collected from nine locations including Anaheim (Orange County), Chula Vista (San Diego County), Eureka (Humboldt County), Fresno (Fresno County), Gardena (Los Angeles County), Ontario (San Bernardino County), Modesto (Stanislaus County), Redding (Shasta County), and San Rafael (Marin County). Four of the nine sites were the same as the 2010 study (Anaheim, Eureka, Fresno, and San Rafael). Researchers recruited over 1,000 drivers during weekend nighttime hours to provide oral fluid specimens voluntarily. Cannabis use throughout the state was again different, depending on geographical location.

The difference in marijuana prevalence in drivers between 2007, 2010, and 2012 will be presented. The effect of medical marijuana availability and potential decriminalization of marijuana in the state of California will be discussed.

Oral Fluid, Marijuana, Driving

K49 Can Oral Fluid Cannabinoid Testing Differentiate Cannabis Smoking From Intake of Oral THC and Oromucosal Sativex® Administration?

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After attending this presentation, attendees will be informed about cannabinoid disposition in Oral Fluid (OF) after a single oral Δ^9-tetrahydrocannabinol (THC) or Sativex® oral mucosal administration. Dronabinol, synthetic THC, is approved in the U.S. for treating anorexia caused by AIDS and chemotherapy-associated nausea/vomiting. Sativex®, a cannabis oromucosal spray containing approximately 1:1 THC and cannabidiol (CBD), is in phase III trials for cancer pain in the U.S. and approved for multiple sclerosis symptoms in several European countries, Canada, and New Zealand. While several studies investigated OF cannabinoid pharmacokinetics following cannabis smoking, there is only one with multiple oral THC doses and none with Sativex®. This presentation characterizes OF cannabinoid time-course profiles, windows of detection, and cannabinoid ratios after the oral and sublingual drug delivery routes. Differences from cannabis smoking were evaluated for possible approaches to identifying relapse and compliance with cannabinoid pharmacotherapy.

This presentation will impact the forensic science community by providing the first data defining cannabinoid disposition in oral fluid after single-dose medicinal cannabinoid products. These data will improve interpretation of oral fluid cannabinoid testing and aid in formulating policy and legislation for oral fluid testing and in effectively managing patients undergoing cannabinoid pharmacotherapy.

Methods: Fourteen cannabis smokers (aged 19 – 43 yrs, 79% male, 64% African American) provided written informed consent for this double-blind, double-dummy, within-subject, Institutional Review Board-approved study. The participants resided on a closed research unit at least 10h prior to each drug administration. Five or 15mg synthetic oral THC; two (low dose, 5.4mg THC, and 5.0mg CBD) or six (high dose, 16.2mg THC, and 15.0mg CBD) actuations of Sativex®; or placebo oral THC and six placebo Sativex® actuations were administered in random order. Dosing sessions were separated by at least five days. OF specimens were collected with the Quantisal™ collection device, 0.5 hr before and 0.25, 1, 4.5, 7.5, and 10.5 hr after dosing initiation. THC, CBD, cannabinol (CBN), 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THCCOOH) were quantified by 2D Gas Chromatography/Mass Spectrometry (GC/MS). If analyte concentrations exceeded the upper limit of linearity, participants' OF specimens were diluted with drug-free OF-Quantisal™ buffer mixture. Limits of quantification were 0.5ng/mL for THC, CBD, and 11-OH-THC, 1ng/mL for CBN, and 7.5pg/mL for THCCOOH.

Results: After oral THC, OF THC decreased over time from baseline concentrations ≤20.5ng/mL; concentrations were not significantly different from those after placebo, reflecting residual THC excretion from previously self-administered smoked cannabis. CBD and CBN were only detected in three specimens with concentrations ≤1.1ng/mL. After Sativex®, THC, CBD and CBN increased greatly with peak concentrations of 266 – 11,424, 196 – 12,120, and 9.5 – 560ng/mL (low dose, respectively); 1,323 – 18,216, 1,552 – 18636, and 74 – 22,32ng/mL (high dose, respectively) occurring at 0.25 – 1 hr, except one CBD at 4.5 hr. After low- and high-dose Sativex®, all specimens were positive for THC and CBD until 10.5 hr post-dose with concentrations 1.0 – 92.0 and 0.5 – 131ng/mL, respectively. After low- and high-dose Sativex®, 43% and 79% of specimens, respectively, were CBN-positive for 10.5 hr with concentrations ≤6.9ng/mL. Median (range) CBD/THC and CBN/THC ratios were 0.82 – 1.34 (0.27-2.26) and 0.04 – 0.06 (0.01-0.52), respectively, over 0.25 – 10.5 hr. In comparison, median (range) CBD/THC and CBN/THC ratios after smoking a single 6.8% THC cigarette were 0.04 – 0.05 (0.03-0.09) and 0.07 – 0.08 (0.04 –0.15), respectively, within 0.25 – 6 hr post dose. OF THCCOOH concentration changes over time were less evident and significantly masked by baseline concentrations in all dosing sessions. THCCOOH/THC ratios were <4pg/ng for 4.5 and 1 hr post Sativex® and smoked cannabis, respectively, while ratios were never below 4pg/ng after oral THC and placebo. THCCOOH/THC ratios increased over time in each dosing session.

Conclusions: Oral THC and Sativex™ administered in low and high dosages produced OF cannabinoid disposition different from those after smoked cannabis; THC, CBD, and CBN were rarely detected after oral THC while Sativex® generated high CBD/THC ratios. Low THCCOOH/THC ratios suggest recent Sativex® and smoked cannabis exposure. Study results indicate that relapse to smoked cannabis during oral THC pharmacotherapy for cannabis dependence should be evident with OF cannabinoid monitoring. In contrast, compliance with Sativex® pharmacotherapy should be clearly apparent by the high OF CBD/THC ratio as compared to that following cannabis smoking; however, additional research is needed to determine if relapse to cannabis smoking can be identified during Sativex® pharmacotherapy, as the high OF CBD/THC ratio after Sativex® may not be altered sufficiently to identify single smoked cannabis episodes. Interpretation of OF cannabinoid tests will be improved by these data, the first defining OF cannabinoid disposition after single-dose medicinal cannabinoid products. These data also

are valuable for formulating policy and legislation for OF testing, and for effectively managing patients undergoing cannabinoid pharmacotherapy.

Oral Fluid, Cannabinoids, Cannabis

K50 Current Research Initiatives in Toxicology at the National Institute on Drug Abuse

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After attending this presentation, attendees will be able to describe new research from Chemistry and Drug Metabolism (CDM), detailing National Institute on Drug Abuse (NIDA) findings on urinary cannabinoid excretion, oral fluid cannabinoid stability, Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) confirmation of urinary synthetic cannabinoids, and performance impairment and blood THC concentrations in driving cases.

This presentation will impact the forensic science community by revealing CDM investigations of illicit drug agonists, antagonists, and drug dependence treatment pharmacotherapies. Controlled drug administration studies in drug users were conducted under Institutional Review Board-approved protocols and Investigational New Drug applications from the Food and Drug Administration.

CDM investigates the pharmacodynamics and pharmacokinetics of illicit drug agonists and drug dependence pharmacotherapies. Phase I controlled drug administration studies are conducted in drug users under Institutional Review Board-approved protocols and Investigational New Drug applications from the U.S. FDA. Recently, this study focused on mechanisms of action of cannabinoid agonists, development of evidence-based drug policy, and legislation for oral fluid testing and emerging designer drugs. In this presentation, new research findings are shared on urinary cannabinoid excretion, oral fluid cannabinoid stability, LC/MS/MS confirmation of urinary synthetic cannabinoids, and performance impairment and blood THC concentrations in driving cases.

Twelve chronic frequent and nine occasional cannabis users smoked one 6.8% Δ^9-tetrahydrocannabinol (THC) cigarette. Cognitive, subjective, psychomotor and physiological responses, and urinary cannabinoid pharmacokinetics were characterized. THC, cannabidiol (CBD), cannabinol (CBN), 11-hydroxy-THC (11-OH-THC), 11-nor-9-carboxy-THC (THCCOOH), THC-glucuronide, and THCCOOH-glucuronide concentrations were simultaneously quantified by LC/MS/MS and normalized to urine creatinine. THCCOOH (frequent N=12; occasional N=6), THC-glucuronide (frequent N=12; occasional N=9), and THCCOOH-glucuronide (frequent N=12; occasional N=9) were identified; THC, 11-OH-THC, CBD, and CBN were not detected. Highest concentrations (ng/mg creatinine) in frequent and occasional cannabis smokers, respectively, were: THC-glucuronide 3.5-60.7 and 3.0-35.1; THCCOOH 2.7-17.2 and 0-7.2; and THCCOOH-glucuronide 146-548 and 20.8-298. Concentration-time curves for the excretion of urinary cannabinoids and the presence of potential markers for recent cannabis smoking will be presented.

Analyte stability is critical for interpreting drug concentrations, although there are few data on cannabinoid stability in oral fluid, an important new drug testing matrix. Cannabinoid stability in authentic oral fluid collected with the StatSure® and Oral-Eze® collection devices after controlled smoking of one 6.8% THC cigarette was evaluated. Stability pools were prepared for each participant (n=16) by combining oral fluid collected in the first 13.5 hr. Pools were aliquoted into polypropylene cryotubes and stored at room temperature (RT), 4°C, or -20°C. Baseline specimens were quantified within 24 hr, and the remaining aliquots analyzed after one week at RT and 4°C, and four weeks at 4°C and -20°C. Specimens were considered stable if concentrations were within ± 20% of baseline. Specimens collected with the Oral-Eze® and StatSure® devices were stable for THC and THCCOOH at 4°C for one week and one month, while after longer frozen storage a small number of specimen concentrations increased or decreased more than ± 20%. Mean THC and THCCOOH oral fluid concentrations were 93.0 – 96.7% of target after refrigeration for one or four weeks, and 89 – 117% (THC) and 81 – 110% (THCCOOH) for one month frozen. Elution and stabilizing buffers in oral fluid collection devices help maintain cannabinoid concentrations in oral fluid, as well as improving cannabinoid recovery from the collection pad.

Synthetic cannabinoids are an important new designer drug class. Assays are needed to identify these drugs in human urine. An LC/MS/MS method was developed for the qualitative confirmation of ten synthetic cannabinoids (JWH-018, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, JWH-250, AM 2201, and RCS-4) and their hydroxyalkyl, hydroxyindole, and carboxy metabolites in human urine. Specimen preparation includes hydrolysis and protein precipitation, followed by monitoring of a single MRM transition in a survey scan that triggers an enhanced product ion (EPI) scan at three different collision energies. This information-dependent acquisition experiment is conducted on an ABSciex 5500 QTrap. Qualitative results from several hundred authentic urine specimens will show prevalence of parent and metabolites and metabolite patterns.

Finally, this latest investigation on cannabis effects on driving is presented. In collaboration with other toxicologists, driving under the influence of drugs (DUID) cannabis cases were compiled and analyzed and police reports on apprehended drivers under the influence of cannabis and blood THC concentrations were evaluated. Representative individual case reports with varying THC concentrations are presented, as well as aggregate/summary statistics. The case reports focus on cannabis-only cases, to avoid complications imposed by polypharmacy.

Urine Cannabinoids, Oral Fluid Stability, Designer Drugs

K51 Detection and Quantification of Antidepressants in Aqueous Matrices

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The goal of this presentation is to delineate the process of developing a detection method capable of simultaneously identifying and quantifying antidepressant compounds in aqueous matrices using a Liquid Chromatograph/Tandem Mass Spectrometer (LC/MS/MS), the determination of quantitative differences between detection methods, the effect of stationary phase composition on the separation of analytes, and applications to wastewater samples pursuant to a relevant EPA method.

This presentation will impact the forensic science community by showing how the methodologies and results presented will provide widely applicable LC/MS/MS method for the detection of small molecules in a variety of aqueous matrices, including wastewater, blood, and urine. The development process can be adapted to produce a quantitative screening method for any compounds of interest suitable for LC/MS/MS identification.

Antidepressants are psychiatric medications that are taken with the intent to alleviate mood disorders. These drugs and their metabolites enter the environment as a byproduct of use, and may pose a danger to the health of humans and the environment. In the U.S., there are few regulations concerning the discharge, fate, and transport of such pharmaceuticals, many of which remain biochemically active after passing through current treatment processes. An important first step toward developing proper regulation is the creation of a selective and sensitive detection method in relevant matrices.

This presentation will discuss the methodology developed for the detection and identification of a variety of commonly used antidepressant drugs and their metabolites. High Pressure Liquid Chromatography (HPLC) on fused core silica phases is used for the separation of analytes, and a triple-quadrupole mass spectrometer is employed using both Scheduled Multiple Reaction Monitoring (s-MRM) and Information Dependent Acquisition (IDA) detection methods with Electrospray Ionization (ESI). Sample preparation is performed in accord with EPA method 1694, using solid-phase extraction with an Oasis® HLB cartridge suitable for retention of acidic, basic, and neutral compounds.

A library containing reference spectra was created using direct syringe pump infusion of standards of each antidepressant in methanol. Generalized parameters capable of ionizing and fragmenting each compound were optimized and compiled. The composite method was used to create and assess the efficacy of identifying and quantifying components of a mixture using both SMRM and IDA.

Preliminary testing was performed on a C18 column, the currently most-used column for analysis of antidepressants. The variance of s-MRM, which scans for analytes at specified expected retention times, was determined to be within acceptable limits to provide reproducible results. Information Dependent Acquisition, a method that scans using s-MRM and produces additional library matching spectra for analytes with intensities over a certain threshold, supposedly at a cost of quantitative reproducibility. The difference in quantification between the two methods was determined using labeled analogues of each compound as internal standards.

The optimized detection method was then applied to chromatographic separations using different stationary phase compositions including C8, C18, phenyl-hexyl, and amide. The goal was to determine how chemical interactions between analytes and the column influence separation and analysis. Parameters such as efficiency, reproducibility, and selectivity were considered in method optimization. Retention times, elution order, and peak shapes were compared when possible.

Antidepressant, LC/MS, Wastewater

K52 ∆9-Tetrahydrocannabinol, 11-Nor-9-Carboxy-Tetrahydrocannabinol, Cannabidiol, Cannabinol, and 11-Hydroxy-Tetrahydrocannabinol in Oral Fluid Following Controlled, Smoked Cannabis in Frequent and Occasional Smokers

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After attending this presentation, attendees will understand pharmacokinetic differences in Oral Fluid (OF) concentrations between chronic frequent and occasional cannabis smokers.

This presentation will impact the forensic science community by improving interpretation of oral fluid test results for different populations of cannabis smokers and may suggest recent cannabis use by selecting different analytes and cutoff concentrations.

In a previous study, additional cannabinoid analytes and cutoff concentrations were proposed to reduce the possibility of a positive cannabinoid OF test from environmental cannabis smoke contamination; however, the data only included chronic frequent cannabis smokers.[1] The objectives of the present study were to compare pharmacokinetic data from chronic frequent and occasional cannabis smokers, and to evaluate different cannabinoid analytes and cutoff concentrations to distinguish recent smoking from residual excretion in these populations.

This study consisted of healthy, 18 – 45-year-old cannabis smokers, who used cannabis at least four times per week (frequent smokers) or a maximum of two times per week (occasional smokers), provided written informed consent for this Institutional Review Board-approved study, and resided on the secure research unit for two days. OF specimens were collected with the Statsure™ device, 14 hr and 1 hr before and up to 30 hr after *ad libitum* smoking of a 6.8% ∆9-Tetrahydrocannabinol (THC) cigarette. Specimens were analyzed within 24 hr following collection. THC, 11-nor-9-carboxy-THC (THCCOOH), cannabidiol (CBD), cannabinol (CBN), and 11-hydroxy-THC (11-OH-THC) were quantified by 2D-GC/MS. Limits of quantification (LOQ) were 0.5 ng/mL for THC, CBD, CBN, and 11-OH-THC, and 15pg/mL for THCCOOH.

Eighteen subjects (eleven chronic frequent and seven occasional cannabis smokers) provided 306 OF specimens. Fourteen hours before smoking, all chronic frequent smokers' OF tested positive for THC (range 6-396.5ng/mL) and THCCOOH (23-124pg/mL), whereas all occasional smokers' OF specimens were negative for both analytes. One hour prior to dosing, nine chronic frequent smokers' OF specimens were still positive for THC (range 0.9 to 7ng/mL), while THCCOOH was always measurable in this population (range 2.0 – 13.6pg/mL).

No significant differences ($p>0.05$) in THC concentrations were observed 2h after cannabis smoking between chronic frequent and occasional smokers. OF THC concentrations were highly elevated for 2h with medians (range) of 517.3ng/mL (113.8 – 6,508), at 0.5 hr, 238.5ng/mL (28.4 – 6,362) at 1 hr and 72.5ng/mL (7.5 – 350.5) at 2 hr for the chronic frequent smokers. Medians (range) in occasional smokers were 481.8ng/mL (84.5 – 1471.3) 0.5 hr after smoking, 82.8ng/mL (48.4 – 561.5) at 1 hr, and 69.3ng/mL (23.4 – 213.7) at 2 hr. Eight of eleven chronic frequent smokers' OF specimens were still THC-positive at 30h (0.6 – 2.2ng/mL), whereas only one of seven occasional smokers was positive (0.5ng/mL). OF THCCOOH concentrations showed large differences between chronic frequent and occasional smokers at all time points: Results showed 83% of all specimens from occasional smokers were negative (all medians equal to 0) and 95% of OF specimens from chronic frequent smokers were positive. For both groups, CBD and CBN concentrations were maximal 0.5 hr after smoking, decreasing rapidly over time. The last positive OF CBD occurred 10.5 hr after smoking in two chronic frequent smokers (0.5 – 0.6ng/mL) and at 6 hr for one occasional smoker. For CBN, four chronic frequent and one occasional smokers' OF were still positive at 13.5 hr (0.6 – 1 and 0.6ng/mL, respectively). 11-OH-THC was not present in OF except when THC concentrations were greater than 1,000ng/mL.

At a cutoff concentration of 2ng/mL THC, proposed by the Substance Abuse and Mental Health Services Administration, 72% and 71% of chronic frequent and occasional smokers were positive for 21 hr and 10.5 hr, respectively.

These findings improve interpretation of cannabinoid OF concentrations in the workplace, cannabis dependence treatment, motor vehicle accidents, and doping in sports cases.

Reference:

1. Lee D, Schwope DM, Milman G, Barnes AJ, Gorelick DA, Huestis MA. Cannabinoid disposition in oral fluid after controlled smoked cannabis. Clin Chem. 2012;58:748-56.

Oral Fluid, Tetrahydrocannabinol, Cannabinoids

K53 Laboratory Based Evaluation of Commercially Available Oral Fluid Testing Devices

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After attending this presentation, attendees will be able to assess sensitivity and specificity, and detection thresholds of several commercially available oral fluid testing devices designed for use in the field, as well as issues with respect to their readability, robustness, and ease of use.

This presentation will impact the forensic science community by highlighting factors to be considered when selecting field-based testing devices for the presence of drugs in oral fluid.

Oral fluid is increasing in popularity as a biological matrix for drug testing in the workplace, probations and parole, and traffic law enforcement.

Three devices were selected for comparison with the Drager® DT5000 7-panel, based on their price, general availability, and advertised ease of use. The devices evaluated were the Oral Fluid 6 Drug Test (Oral Q®), Alere iScreen®, and Xalex™. Nine blind controls containing a total of 12 drugs representing the classes of amphetamines, MDMA, opiates, benzodiazepines, PCP, cocaine, THC, methadone, oxycodone, and dextromethorphan were prepared in synthetic oral fluid. An open positive (100ng/mL) and drug-free oral fluid negative control were also used.

The concentrations of the target analyte were selected so evaluations could be made below the listed cutoff concentration, near the cutoff concentration, and significantly above the advertised cutoff of the device.

Each device was evaluated in triplicate for each control group with the results being independently verified by two different individuals. The testing protocol used was specific to the device, following a protocol based on the device instructions. In devices with a sorbent sponge, the sponge was saturated with the oral fluid control mixture and subsequently tested as directed. After the verification of the results, the performance of each device was evaluated by drug class and how the device performed around its stated cutoff concentration. For each device, the sensitivity, specificity, and accuracy were assessed.

Positive results were scored as true positives if the analyte was present in the control, irrespective of its concentration. With all negative results, the concentration in the control was compared to the manufacturer's cutoff concentrations and determined if the result was a true positive or true negative relative to that cutoff.

The Drager® DT5000 was an instrumented test with an electronic analyzer generating a printed result. The remaining devices were visually read. These three had cannabinoid tests that were targeted to carboxyTHC which is known to be excreted at very low concentrations in oral fluid. The Xalex™ and Alere iScreen6 did not give a positive cannabinoid result at 100ng/mL of THC. The OralQ gave false positive results for cannabinoids in every negative control. The Drager® DT5000 did not detect the presence of THC at the positive control concentration of 7ng/mL, in spite of its published cutoff of 5ng/mL. Controls at 15ng/mL all tested positive.

The Drager® DT5000 had lower cutoffs for benzodiazepines, methamphetamine, and opiates. The OralQ® had an elevated cutoff for benzodiazepines at 50ng/mL. The Xalex™ and Alere iScreen6® devices did not include a benzodiazepine test. The Drager® DT5000 gave positive results for benzodiazepines at its advertised cutoff of 15ng/mL.

The sensitivity and specificity results for the Xalex™ and iScreen6® did not include scoring from the THC panel because the target analytes were THC metabolites, which would not be expected at the advertised cutoff in oral fluid. Absent this consideration, the Xalex™ device had sensitivity, specificity, and accuracy of 100%, and the Alere iScreen6® had 95% sensitivity, 93% sensitivity, and 94% accuracy. The OralQ® had the lowest sensitivity at 65%, specificity of 86%, and accuracy of 75%. It generated 16 false negative results relative to its advertised cutoffs across several drug classes. The Drager® DT5000 had 97% sensitivity, 100% specificity, and 98% accuracy.

Based on this initial evaluation, it was concluded that the Drager DT5000 gave the best overall performance and lacked the issue of subjectivity in reading the test strips. This laboratory-based assessment, however, indicated it had higher sensitivity for THC than advertised. Additional devices are in the process of being evaluated.

DUID, Oral Fluid, Field Test

K54 Simultaneous Analysis of Opiates and Acetaminophen With Noscapine Monitoring

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After attending this presentation, attendees will learn about a novel liquid extraction solvent and acquisition cycle for determining opiates in forensic toxicology specimens. This extract is suitable for analysis by Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) to measure various common opiates, along with acetaminophen as a co-compound and monitoring of noscapine as an alkaloid contaminant from illicit morphine preparations. The procedure is a robust and sensitive method for routinely evaluating blood, stomach, urine, and vitreous humor for evidence of opiate use and abuse. The audience will also learn about the opiate levels commonly found in postmortem death investigations, DUI suspects, and sexual assault victims. In addition, heroin deaths involving related concentrations of 6-monoacetylmorphine, codeine, and morphine will be reconciled with the presence of noscapine.

This presentation impacts the forensic science community by distinguishing a new way to analyze opiates with advantages on enhanced recovery, preparation economy, time savings, and signal stability. Since implementing this procedure over the last year, not one analytical run failed to meet acceptance criteria. Sample volumes have been reduced by half, turnaround times have been decreased, and more information is obtained without a separate extraction for acetaminophen analysis. These benefits raise services for clients in the community and help to achieve goals for laboratory accreditation.

Opiates are an important category in forensic toxicology for their prevalence in impaired drivers and overdose deaths. Another factor often overlooked is that acetaminophen is a toxicologically significant drug frequently compounded with hydrocodone tablet preparations. Although Solid Phase Extraction (SPE) purifications are a useful way of obtaining clean drug extracts for instrumental analysis, acetaminophen recovery suffers greatly. A multiple-targeted analysis of opiates consisting of morphine, hydromorphone, oxymorphone, codeine, oxycodone, 6-monoacetylmorphine, acetaminophen, and hydrocodone combined with noscapine monitoring using a modified Liquid-Liquid Extraction (LLE) has been developed. The simple LLE method utilizes a mixture of organic solvents consisting of isopropyl alcohol, isoamyl alcohol, and 1-chlorobutane for extraction to yield enhanced recovery that saves money and time.

In the LC/MS/MS acquisition method, two separate mass spectroscopy segments were utilized to improve the detection sensitivity, while short dwell times were used for acetaminophen transitions to lower sensitivity in adjusting for its relatively high concentration scale. The opiates follow a linear curve fit from 10ng/mL to 2,000ng/mL with a corresponding curve range of 1mg/L to 200mg/L for acetaminophen. The 15-minute chromatography gradient cycle allows for clean separation of all analytes, especially morphine and hydromorphone as well as codeine and hydrocodone, which are isobaric pairs that can interfere with each other in qualitative and quantitative confirmations.

This method has many benefits for the toxicology laboratory at many levels. Extraction analysts find the process to be faster and easier than SPE protocols. Instrument data processors report stronger responses with sharp peaks, linear curve fits, and quality controls in good agreement with expected outcomes. Laboratory managers realize successful sequential analytical runs decrease turnaround time, which reduces the burden on consumable budget, and provides more data using less specimen. Overall, this method has been validated as a superior process for routine forensic toxicology opiate analysis.

Opiates, LC/MS/MS, Toxicology

K55 Method Development and Validation for the Analysis of Cannabinoids in Meconium Samples

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After attending this presentation, attendees will be able to compare different solid-phase extraction methods for the cleanup of meconium samples prior to Three-Dimensional/Gas Chromatography/Mass Spectrometry (3D/GC/MS) analysis.

This presentation will impact the forensic science community by providing perspectives on how to assess and improve methods for extracting, isolating, and measuring cannabinoids and their metabolites from meconium.

The purpose of this project was to develop an extraction method and instrumental parameters for GC/MS and 3D/GC/MS methods for the analysis of tetrahydrocannabinol (THC), 11-OH-THC, and THC Carboxylic Acid (THCC) that meet laboratory requirements for accuracy and precision and is cost and time efficient. The project resulted in development of an improved screening for the presence of drugs in meconium samples in aid of diagnosing and detecting marijuana use by the mother during gestation.

Collectively, the components of the marijuana plant, *Cannabis sativa*, are known as cannabinoids, and have a variety of pharmacological effects in humans including, but not limited to, analgesia, appetite suppression, hypertension, euphoria, and suppression of nausea. The main active component, THC, is broken down by the liver into a variety of oxidized metabolites. The major route of metabolism is hydrolysis of THC at carbon-11 to form 11-OH-THC which is further oxidized to form 11-Nor-carboxy-THC (THCC). These three components are the major focus of most marijuana testing in human bodily fluids. Prenatal exposure of THC is thought to possibly have detrimental effects, including effects on the systems involved in emotions and maturation. Animal studies have shown that rat pups prenatally exposed to THC could have long-lasting neurological effects. Many drug court and monitoring programs require the mother to abstain from marijuana use while pregnant to avoid exposure of the developing fetus to cannabinoids, and methods are needed to monitor their compliance with those orders.

Comparisons of several variables for the isolation of cannabinoids from meconium were performed, including the comparison of two different brands of THCC-specific Solid Phase Extraction (SPE) columns, Agilent™ and Strata X™, the length of incubation while hydrolyzing the samples, the effect of homogenizing the meconium sample before extraction, the wash solvents used, and the effects of the polarity of the GC/MS columns on which the samples were run.

The significance of these variables was evaluated by performing the same extraction with only the variable in question being altered. The general extraction method includes adding 0.25g of samples to phosphate buffer pH 7.0 and adding 25µL of 12M potassium hydroxide to hydrolyze the samples. After hydrolysis, the samples were neutralized using hydrochloric acid. The neutralized samples were then treated with acetonitrile precipitation and centrifugation before pouring the supernatant onto the SPE columns. The columns were the washed, eluted, and the extract dried down before derivatization with BSTFA.

From the comparison between the two SPE columns, it was determined that the Strata X™ columns had a better recovery of THCC with the extraction method used while the Agilent™ columns had a better 11-OH-THC recovery. The percent recovery of THCC and 11-OH-THC while using the Agilent™ columns was determined to be 91% and 66.5%, respectively. The Strata X™ columns resulted in a 95% and 56% recovery for THCC and 11-OH-THC, respectively. The incubation-time evaluation led to the conclusion that while there was little change in recovery from commercially available control samples (ElSohly Labs), an authentic THC-positive meconium control sample showed significantly increased abundance of free THCC at 30 min incubation at 60°C.

Applying the optimized extraction method using deuterated internal standards for all three analytes generated calibration curves with R^2 values greater than 0.998.

The study concluded that SPE analysis of meconium samples gave cleaner extracts than liquid/liquid extraction, that hydrolysis improved recovery of the drug from the sample, and that combined with GC/MS, SPE produced calibration curves that met laboratory requirements.

Cannabinoids, Meconium, GC/MS

K56 Effect of Ethanol on Succinyl Semialdehyde Dehydrogenase—Implications for Exacerbation of GHB Toxicity

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After attending this presentation, attendees will gain a greater understanding of the central nervous system, its endogenous inhibitory neurotransmitters and their metabolism, and the effects of ethanol on part of that metabolic process.

This presentation will impact the forensic science community by offering a mechanistic explanation for one aspect of the combined effects of ethanol/GHB co-ingestion. This knowledge will help forensic pathologists and toxicologists evaluate and interpret drug results in DFSA cases where both ethanol and GHB are determined to have been present.

Drug Facilitated Sexual Assault (DFSA) cases routinely involve the use of central nervous system (CNS) depressant agents such as benzodiazepines, barbiturates, and more recently, γ-hydroxybutyrate (GHB). A common mechanistic basis for the actions of these agents is an effect on GABA-nergic inhibitory neurotransmission. GABA (γ-amino butyric acid) is the primary inhibitory neurotransmitter in the CNS, functioning as a post-synaptic ligand-gated chloride channel agonist (GABA receptor agonist). Activation of the GABA receptor by either endogenous GABA, or by xenobiotics, results in the influx of chloride ion into the post-synaptic neuron, resulting in a hyperpolarization (inhibition) of that neuronal membrane. Removal of GABA from the synaptic space following the neurotransmission event is a function of uptake and catabolism of GABA by astrocytic cells in proximity to the synapse rather than re-uptake directly into the pre-synaptic neuron. Succinyl Semialdehyde Dehydrogenase (SSADH) is a central enzyme in the oxidative degradation of GABA and GHB, converting their common oxidative metabolite, succinyl semialdehyde (SSA), to succinate as an end product. SSA is produced directly from GABA by an enzyme-catalysed transamination (with alpha-keto glutarate, (αKG)), and from GHB by a GHB-dehydrogenase (GHBDH)-catalysed oxidation. GHB dehydrogenase is a cytosolic

enzyme reducing NAD^+ as a cofactor, while SSADH and the transaminase are mitochondrial enzymes, with SSADH reducing $NADP^+$ as a cofactor.

Ethanol is commonly found in DFSA cases, either alone or in combination with other CNS depressants, including GHB, and its presence may have an impact on the interpretation of drug findings in such cases. Ethanol has been shown to exacerbate the effects of GHB; however, a mechanistic basis for that effect has not been demonstrated. It has been hypothesized that one consequence of alcohol ingestion in the body is an inhibition of SSADH by both ethanol and its oxidative metabolite, acetaldehyde, because of the structural homology between ethanol, acetaldehyde, and carbons 3 and 4 of SSA. Inhibition of SSADH would be expected to increase the effective half-life of GABA in the body, with the consequential increase in background GABA concentration, and GABA-mediated CNS depressant activity. Initial experiments with a combined enzyme system consisting of GABA-αKG transaminase/SSADH indicated that ethanol inhibited enzyme activity at a concentration equivalent to 0.4 g/dL, but did not do so appreciably at a concentration equivalent to 0.1g/dL, suggesting that any such effect of ethanol on SSADH would only be a factor in significant alcohol ingestions. Kinetic evaluation of initial reaction rates by UV spectrophotometry (monitoring generation of NADPH) indicated that ethanol affected SSADH rather than the GABA-αKG transaminase. Substrate-velocity experiments indicated that SSADH in the preparation had a Michaelis constant (K_m) for SSA of 49 μM in the absence of ethanol, and 61 μM in the presence of 0.4g/dL ethanol, as determined by Lineweaver-Burke plot. Maximal velocity (V_{max}) of the enzyme was unaffected by the inclusion of ethanol, a pattern consistent with competitive inhibition.

Based on the effect of ethanol on SSADH, it is suggested that the ingestion of alcohol in the body would, in a concentration-dependent manner, inhibit SSADH, thereby decreasing the rate of GABA- and/or GHB-derived SSA oxidation, and potentially increasing both the half-life of endogenous GABA and exogenous GHB. This effect may play a contributory role to the CNS depressant consequences of significant ethanol ingestions and combined ethanol-GHB exposures, such as could be seen in some DFSA cases.

Ethanol, GHB, GABA

K57 Incomplete Recovery of Codeine in Urine Using Common Enzymatic Hydrolysis Procedures

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The goal of this presentation is to inform attendees about the effectiveness of various commonly used hydrolysis techniques and conditions for the hydrolysis of opiates, stressing the incomplete hydrolysis of codeine following common enzymatic hydrolysis procedures.

This presentation will impact the forensic science community by demonstrating that choosing the right combination of hydrolyzing agent and hydrolysis conditions is critical to accurate results and leads to significant improvement in recovery of opiates from urine samples.

Methods: Opiates included in this method are: morphine, hydromorphone, codeine, and hydrocodone. Deuterated analogues of all four analytes are used as the internal standards.

Extraction: Authentic urine samples were hydrolyzed using β-glucuronidase from *Escherichia coli and Helix-Pomatia* for 3 hr and 16 hr each. Samples were spiked with internal standard, centrifuged and supernatant was diluted with mobile phase before injecting on the column. In separate experiments, the amount of enzyme added was doubled to evaluate optimal concentration of the enzyme for efficient hydrolysis. In addition, one set of samples was hydrolyzed using acid hydrolysis with 0.1N HCl and the results were used as the reference (100% recovery) to evaluate recovery from different enzymatic procedures.

Analysis: Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) analysis was performed in Electronic Spray Ionization (ESI) mode by Multiple Reaction Monitoring (MRM) using a 3200 triple quadrupole mass spectrometer connected to a Shimadzu prominence HPLC system. Separation was achieved on an ultra II biphenyl 5µ column (50 X 2.1mm). Mobile phases were 0.1% formic acid and 0.2% ammonium formate in de-ionized water (A) and in acetonitrile with 2% water (B). All analytes were eluted within four minutes. Two ion transitions for each analyte; morphine (286/152, 286/128), hydromorphone (286/185, 286/157), codeine (300/152, 300/115), hydrocodone (300/199, 300/128), and one ion transition for each internal standard; morphine-D6 (292/152), hydromorphone-D3 (289/185), codeine-D6 (306/165), and hydrocodone-D6 (306/202) were monitored.

Results: The procedure was applied to 50 authentic urine specimens previously tested positive for two or more analytes using acid hydrolysis and GC/MS. Results showed that efficient hydrolysis is essential to the optimum recovery of all analytes. β-glucuronidase from both *H. Pomatia* and *E. Coli* were not able to cleave codeine glucuronides efficiently and recovered only 25% and 50% of the free drug after 3 hr and 16 hr hydrolysis time, respectively. On the contrary, 100% recovery was achieved for hydrocodone after 3 hr with both *H. Pomatia* and *E.Coli*. Average morphine recovery was 84% with *H. Pomatia* at 3 hr and 100% after 16 hr of incubation. *E. Coli* recovered 77% and 89% morphine at 3 hr and 16 hr, respectively. Average hydromorphone recovery with *H. Pomatia* was 80% after 3 hr and 95% after 16 hr. *E. Coli* recovered only 41% hydromorphone at the end of 3 hr and 58% after 16 hr of incubation. Doubling the amount of enzyme did not improve the recovery for any of the opiates.

Conclusion: Acid hydrolysis for opiates has been commonly used with GC/MS analysis in the past. With the advancement of instrumentation, LC/MS/MS is gaining popularity in the clinical and forensic labs and enzymatic hydrolysis is the preferred method for releasing the free drugs. Post-enzymatic hydrolysis specimens can simply be diluted and injected on the column, eliminating the need for time-consuming extractions. It is essential, however, to optimize the hydrolysis conditions for the opiate glucuronides specific to each source of β-glucuronidase. Codeine glucuronide is the most difficult to cleave and only 50% of the drug was recovered in free form after 16 hr of hydrolysis with β-glucuronidase from *H. Pomatia.* In general, the enzyme from *H. Pomatia* performed better than the one obtained from *E. Coli*, under the conditions tested. Although *H. Pomatia* was able to release 100% of the free drug form morphine, hydrocodone, and hydromorphone conjugates in the urine samples at the end of 16 hr (3 hr in case of hydrocodone), it was found to be ineffective in cleaving codeine glucuronide. Further investigation is necessary to find the optimal conditions for enzymatic hydrolysis of codeine. The labs must carefully evaluate the hydrolysis efficiency of various enzymes for opiates and specifically for codeine.

Opiates, Hydrolysis, LC/MS/MS

K58 Sweat as Alternative Matrix to Monitor Buprenorphine Compliance, Opioids, Cocaine, and Tobacco Use in Opioid-Dependent Pregnant Women

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The goal of this presentation is to describe Buprenorphine (BUP), opioids, cocaine, and tobacco prevalence and concentrations in sweat patches from opioid-dependent pregnant women, and to compare their detection in sweat patches, Oral Fluid (OF), and urine.

This presentation will impact the forensic science community by showing how sweat is a good alternative matrix for monitoring drug use in clinical settings.

Introduction: Sweat is an alternative matrix for detecting drug consumption over about seven days, depending upon the time the patch is worn. Sample collection is easy, gender-neutral, and less invasive than for urine collection. However, limited sweat disposition data are available, especially for BUP and for opioid-dependent women.

Objective: To describe BUP, opioids, cocaine, and tobacco prevalence and concentrations in sweat patches from opioid-dependent pregnant women, and to compare detection in sweat patches, OF, and urine specimens over the same period.

Methods: Sweat patches were collected once weekly (n = 121), and OF and urine twice or three times weekly (n = 283) from seven opioid-dependent pregnant women during the 2nd and, primarily, the 3rd trimester, and up to one month postpartum. Sweat was collected with PharmCheck™ sweat patches worn for 6 ± 2.3 days, and OF with the Salivette® collection device. Sweat and OF specimens were analyzed by Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) for BUP, norbuprenorphine (NBUP), methadone, 2-ethylidene-1,5-dimethyl-3-diphenylpyrrolidine (EDDP), cocaine, benzoylecgonine (BE), ecgonine methyl ester (EME), morphine, codeine, 6-acetylmorphine (6AM), heroin, 6-acetylcodeine (6AC), cotinine, and trans-3′-hydroxycotinine (OH-cotinine) (LOQ 1-5ng/patch, 0.5-1ng/mL, respectively). Urine specimens were assayed for cocaine and opiates by immunoassay (cutoff 300ng/mL). Women received 8 – 24mg BUP daily.

Results: BUP was detected in 88% of sweat patch specimens (median 2ng/patch; range 1 – 15.3ng/patch) and NBUP in 37.2% (range 1 – 24.7ng/patch). BUP alone was dectected in 51.2%, along with NBUP in 37.2%. Cotinine was detected in 89.3% (median 159ng/patch; range 8.9 – 1,390ng/patch) and OH-cotinine in 86% (median 52.5ng/patch; range 3.1 – 377ng/patch). Most OF specimens contained both analytes (86%). Methadone from non-prescribed sources was detected in 47.9% specimens (range 1 – 661ng/patch); and EDDP in 14.9% (range 1 – 18.4ng/patch). In 24% of specimens, 6AM was identified (range 1.2 – 180ng/patch), morphine in 23.1% (range 2.3 – 51.3ng/patch), heroin in 14% (range 1.1 – 526 ng/patch), codeine in 9.1% (range 4.5 – 25.1ng/patch), and 6AC in 8.3% (range 1.4 – 17.8ng/patch). Morphine and 6AM were detected alone (5% and 3.3%, respectively), together (5.8%), or in combination with the other analytes (6.6%). For identifying illicit cocaine exposure, cocaine was identified in 88.4% of specimens (median 14ng/patch; range 1 – 5,660ng/patch), BE in 47.9% (range 1.4 – 850ng/patch), and EME in 18.2% (range 8.7 – 567ng/patch). Cocaine was detected alone in 39.7% of cases, cocaine and BE in 30.6%, and the three analytes in 17.4%. Comparing sweat patches and urine, there was an 85.1% concordance for opioids, while for cocaine, only 35.9% agreement was achieved. Sweat patches and OF concordance was 93% for tobacco, 88.6% for BUP, 81.6% for opioids, 61.5% for methadone, and 56.1% for cocaine.

Conclusions: These results offer new information about drug and metabolite concentrations and prevalence in sweat from opioid-dependent pregnant women. Sweat is a good alternative matrix for monitoring drug use in clinical settings.

Sweat, Pregnant Women, Drug Testing

K59 Discovery-Based Analyses of Wastewater Samples for Characterization of Drug Usage

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After attending this presentation, attendees will understand the principles and design of Wastewater Treatment Facilities (WWTFs) and how these locations and wastewater systems in general may be used to determine temporal and spatial trends in the usage of a wide variety of compounds. Monitoring human usage of several categories of controlled substances can yield the information to allow for either targeted enforcement or targeted education. Additionally, the location of possible synthetic operations may be determined through the monitoring of wastewater. This may aid law enforcement in focusing their efforts in a certain region. Additionally, attendees will have a better understanding of appropriate extraction methods used to recover specific analytes from wastewater and also know what steps can be taken to identify and quantify the analytes of interest.

This presentation will impact the forensic science community by providing a mechanism to inform law enforcement and community leaders on community drug habits by the development of a method for monitoring wastewater. Questions such as the following can be answered using this approach: In what areas/neighborhoods are drugs being consumed? What drugs are being consumed? In what volumes are drugs being consumed and in what usage patterns? This could help pinpoint at-risk populations for drug abuse, which may help tailor the drug education curriculum in specified school districts. This could also help facilitate law enforcement in combating areas with identified drug usage.

The information obtained through the monitoring of WWTFs may be used for a variety of purposes. The objective of this research is to determine compounds present in wastewater samples specifically obtained from the Pennsylvania State University wastewater treatment plant, which is being used as a control facility to refine analytical methodology. Rather than beginning with a target compound approach, a discovery analysis approach was chosen to attempt to determine as many compounds as possible prior to any compound list restriction. The difficulty in this approach can be the resulting complexity of the analysis. For this reason, utilization of both Comprehensive Gas Chromatography coupled with Time-Of-Flight/Mass Spectrometry (GC x GC/TOF/MS) analysis and also High Performance Liquid Chromatography coupled with Time-Of-Flight/Mass Spectrometry (HPLC/TOF/MS) analysis were chosen for their inherent ability to characterize these potentially complex samples more successfully compared to other possible techniques. Several categories of compounds were found using this approach in the initial discovery experiments. Specifically, a number of antidepressants (SSRI's and MAOI's), synthetic opioids, and steroids, were found in addition to a number of endocrine disrupting compounds. Additionally, a series of chlorophenyl cyanates were found, which may indicate a chemical synthesis operation, though likely not an illicit drug facility. The determination, through spatially-resolved sampling, of the source will serve as a model for how other synthesis operations could be uncovered and located through the use of this procedure. An example of the complexity of the analysis is revealed through inspection.

Once the discovered compounds are identified and quantified, the ultimate goal is to determine when and where these compounds were introduced into the wastewater system. Employing time-resolved sampling at locations upstream from the WWTF, both the location and the usage patterns were narrowed down. Although a large array of compounds could be identified in the wastewater, this research project will focus particularly on drugs and drug metabolites.

For the discovery phase, samples of wastewater were gathered by "grab" sampling from the Penn State WWTF. Multiple four-liter samples were gathered from each of the following: influent flow, effluent flow, three intermediate stages, and final spray effluent. Following USEPA method 3510, a liquid-liquid extraction process was performed to demonstrate a "baseline" to compare with other extraction methods. A separatory funnel was used for extraction purposes, with methylene chloride as the solvent. Immediately following, the Kuderna-Danish technique was used to concentrate the samples to 1mL. Once the samples had undergone the clean-up process, they were introduced to the analytical systems to identify and quantify the compounds.

The presentation will discuss the methods used to extract, identify, and quantify the analytes of interest. Also, in the discussion, Pharmaceuticals And Personal Care Products (PPCPs) will be brought to the forefront for conversation, as this research project's focus also covers PPCPs as well as emphasizing drugs and drug metabolites.

Analysis, Wastewater, Drugs

K60 Analysis of the Cocaine Metabolite Benzoylecgonine in Wastewater

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After attending this presentation, attendees will gain insight on the utility of sewage epidemiology as a tool in forensic toxicology and the multiple applications of this methodology beyond the forensic science discipline, particularly in regard to toxicological investigations within law enforcement. The presentation will delineate the long-term benefits of this approach to society and law enforcement.

This presentation will impact the forensic science community by contributing an optimized method to forensic toxicology. Sewage epidemiology will reveal comprehensive information on the concentrations of illicit drugs in raw sewage that enables a more precise estimation of their illegal usage, and a relatively quick alternative to gain insight into the toxicological map of a given area. The presentation is geared toward generating interest in developing robust techniques for continuous data generation that can be used to make correlations with crime statistics and create sturdy monitoring tools.

Abuse of illicit drugs is a major problem in society and leads to high morbidity, mortality, and is responsible for many socio-economic problems. Estimates of cocaine consumption are currently obtained from crime statistics, population surveys, and consumer interviews; these estimation methods may not reflect the real extent of cocaine abuse. Another approach that has been used successfully in Europe is sewage epidemiology—a technique based on analysis of urinary biomarkers in sewage. This approach is based on analysis of a stable cocaine metabolite, Benzoylecgonine (BE) in waste water. In humans, cocaine is extensively metabolized to BE by chemical hydrolysis and Ecgonine Methyl Ester (EME) by enzymatic hydrolysis. BE is the major metabolite of cocaine; its presence in urine confirms cocaine abuse. In urine, cocaine can be detected up to 8 hr after use, while BE and EME can be identified for more than 96 hr after cocaine use.

Sewage epidemiology offers an adaptable, alternative method to consistently measure and monitor community drug use. Furthermore, the results from the study can be used to establish a framework for drug use monitoring. The goal of this study was to test the utility of sewage epidemiology in monitoring cocaine metabolite BE in waste water. Influent to the Lubbock (TX) Water Reclamation Plant (LWRP) was tested twice a week to assess weekly variations in cocaine consumption over a five-month period (February 2010 – June 2010). BE was extracted from influent wastewater samples using solid phase extraction and analyzed using Gas Chromatography/Mass Spectrometry (GC/MS). Filtered 500ml influent waste water samples were spiked with deuterated internal standard (BE-d_3) and extracted using Oasis® MCX 60mg SPE cartridges. To determine BE and BE-d_3 in sample extracts using GC/MS, the extracts were first derivitized using N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) to enhance volatility forming the Trimethylsilyl (TMS) derivative of BE or BE-d3. GC/MS analyses was performed using a DB-5MS column for separation and the Mass Selective Detector (MSD) was set to operate under selected ion monitoring mode targeting ion masses of 82, 240, and 361 for BE and 243 and 364 for BE-d_3.

The concentrations of BE derived from the analysis were used to calculate cocaine equivalents deposited in the sewer system through excretion by users. The cocaine equivalents and wastewater daily volumes and flow rates were used to estimate cocaine use by the population. The average daily consumption of cocaine during the study period was estimated at 1,152 ± 147g. Higher cocaine consumption was observed on weekends compared to weekdays. The present study showed that sewage epidemiology is a useful tool to detect BE and subsequently estimate cocaine consumption. The method described is an efficient tool for investigating temporal variations (daily, weekly, and seasonal) at a local level. In addition, this method, along with the ability to sample wastewater at the neighborhood level, could provide a valuable forensic tool for law enforcement.

Cocaine, Sewage-Epidemiology, Toxicological Map

K61 Weeding Analytes Out of Marijuana: The Identification and Quantification of Pesticides in Cannabis Utilizing Comprehensive Gas Chromatography

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After attending this presentation, attendees will understand the principles of analyzing and quantifying cannabis for specific cannabinoids such as Cannabinol (CBN), Cannabidiol (CBD), Cannabichromene (CBC), cannbigerol (CBG), and Delta-9-Tetrahydrocannabinol (THC) using Gas Chromatography with Flame Ionization Detection (GC/FID). They will also understand the concepts and reasons for testing for pesticides contained on cannabis utilizing Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography/Electron-Capture Detectors (GC/ECD) as well as the practical applications for such analyses. Finally, attendees will understand how Comprehensive Gas Chromatography (GCxGC) can be utilized to potentially determine both potency as well as trace organics characterization in a single analysis.

This presentation will impact the forensic science community in a variety of different ways, but loosely falls into two classes: characterization of cannabis as a potential pharmaceutical; and, potentially fingerprinting the trace compounds in cannabis to determine the point of origin. Testing for potency can help determine the identity and abundance of target cannabinoids that have therapeutic qualities. These qualities have been confirmed to relieve pain, control nausea, stimulate appetite, and decrease ocular pressure.[1] With this knowledge, medical marijuana can be grown more effectively by lessening the main psychoactive component, THC, which may cause discomfort in patients, and increasing the target therapeutic cannabinoids.[2] The abundance of THC and other

cannabinoids is affected by a variety of factors including environmental conditions, harvesting periods, and the sex of the plant.[3] Furthermore, many pesticides, fungicides, and insecticides are used to treat the cannabis plant. This is of concern for any person that consumes the material due to the residual toxins that are potentially harmful. Moreover, a study was conducted in 1992 for the United States Drug Enforcement Administration (DEA) which determined that chemical profiles of cannabis samples could be used to locate the geographical origin.[4] However, the system could only eliminate possible sources of origin and therefore had low specificity due to the fact that only cannabinoid constituents were analyzed.[4] By identifying and quantifying the pesticides on the cannabis plant, it may be possible to develop a "chemical fingerprint" relating to compounds used by growers to increase the crop yield. This information may allow law enforcement agencies determine and/or link the source location of the confiscated illicit drug.

One hundred and six different samples of illicit marijuana were analyzed. These were obtained directly from local law enforcement personnel. Samples were initially homogenized, and the finely ground marijuana, weighing approximately 0.2 to 2g, was mixed with 10mL of acetonitrile and 10mL of water in 50mL centrifuge tubes, similar to the QuEChERS extraction procedure developed at the USDA.[5] Water was added to increase the extraction efficiency of more polar pesticides.[6] This solution was spiked with internal standards and pesticides for recovery purposes before soaking for an hour. The solution was shaken for 30 min with a vortex mixer. QuEChERS EN salts were added and the solution was shaken for 1 min.[5] This was followed by a 5 min phase separation utilizing centrifugation.[5] The supernatant was removed and refrigerated.[6] QuEChERS extraction is an efficient method that minimizes organic solvent waste and increases laboratory throughput as compared to more conventional solvent extraction techniques. SPE clean-up followed the extraction step to remove high levels of chlorophyll and organic acids that may interfere with the resulting chromatographic analysis.[6] Sample extract clean-up was performed by cartridge SPE procedures utilizing a 500mg graphitized carbon black/500mg Primary Secondary Amine cartridge, to which MgSO4 was added to the top of the SPE cartridge at approximately half the height of the GCB/PSA bed. The cartridge was rinsed with 20mL of acetone. Then 0.5mL of the sample extract was added, 2.5uL of anthracene (recovery surrogate) was spiked onto the cartridge and carefully mixed by syringe. The solution was eluted with a 3:1 acetone:toluene mixture. The solution was then evaporated with nitrogen at 108°C until it was reduced to approximately 0.3mL. Toluene was added to adjust the final volume to 0.5mL. The samples were analyzed using various GC methods, which will be discussed in detail during the presentation, allowing for the potency analysis and the pesticide fingerprint to be determined in a single GCxGC separation.

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Cannabinoids, GC/MS, GC-FID

K62 Forensic Toxicology Findings in 150 Alleged Cases of Drug-Facilitated Sexual Assault (DFSA) in San Francisco

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After attending this presentation, attendees will understand the wide variety of drugs commonly encountered in DFSA cases and value the usefulness of toxicology testing in such cases.

This presentation will impact the forensic science community by providing valuable information on drug incidence in victims of DFSA and will offer blood reference concentrations of drugs commonly detected in such cases.

Between July 1, 2010, and June 30, 2011, the Laboratory Division of the San Francisco OCME performed toxicologic evaluations in 150 cases of suspected Drug Facilitated Sexual Assault (DFSA). The age of the subjects ranged from 12 to 57 years (mean: 30). The 150 cases were comprised of 129 females, 18 males, and three transgender females. Healthcare providers attending to DFSA victims are trained to collect urine specimens in these types of cases and only collect blood specimens if the alleged crime took place within hours of their examination. Specimens are typically evaluated for ethanol and related volatiles using Gas Chromatography equipped with Flame Ionization Detection (GC/FID), then screened by Enzyme-Linked Immunosorbent Assay (ELISA) for amphetamines, benzodiazepines, cannabinoids, cocaine, methadone, phencyclidine (PCP), ketamine, and opiates/opioids and by Gas Chromatography/Mass Spectrometry (GC/MS) for over 100 other drugs and metabolites including, but not limited to, diphenhydramine, carisoprodol, meprobamate, and γ-hydroxybutyrate (GHB).

Of the 150 cases, 101 had urine only, 17 had blood only, one had plasma only, and 31 had both blood and urine specimens. The 101 cases with only urine pertained to 93 females, five males and three transgender females. Of them, 33 cases had no drugs detected, but 28 were positive for ethanol with a mean ethanol concentration of 0.22% (w/v) and a range of 0.04 to 0.44 % (w/v). It is noteworthy that ethanol is only quantified in urine if the Division is provided with a forensic urine specimen (i.e., a urine specimen obtained about 20 min after the voiding of the urinary bladder; a collection protocol believed to produce urine that is a recent kidney filtrate and which better approximates blood ethanol concentrations). Besides ethanol, the most frequently reported substances in these urine specimens were cocaine/benzoylecgonine (15/18 cases), THC-COOH (14 cases), levamisole (11 cases), diphenhydramine (10 cases), and methamphetamine/amphetamine (8/8 cases). Many other psychoactive compounds were also detected as listed in Table 1.

Table 1: Drugs in Urine-Only Cases

Compound	Frequency
Benzoylecgonine	18
Cocaine	15
THC-COOH	14
Levamisole	11
Diphenhydramine	10
Methamphetamine	8
Amphetamine	8
Cocaethylene	5
Methadone	4
Oxycodone	3
Morphine	2
Codeine	2
MDMA	2
MDA	2
Citalopram	2
Pseudoephedrine	2
Dextromethorphan	2
Sertraline	2
7-Aminoclonazepam	2
Alprazolam	1
Diazepam	1
Nordiazepam	1
Lorazepam	1
6-Monoacetylmorphine	1
Diacetylmorphine	1
Hydrocodone	1
Hydromorphone	1
Bupropion	1
Promethazine	1
Venlafaxine	1
Trazodone	1
Mirtazepine	1
Phencyclidine	1
THC	1
Carbamazepine	1

The 18 cases which had blood products (17 whole blood specimens and one plasma specimen) pertained to 10 females and 8 males. These alleged victims' age averaged 27 years (range: 12 – 41 years). The plasma case was found to be negative. Ethanol was reported in five cases with a mean concentration of 0.11% (w/v) and a range from 0.01 – 0.15% (w/v). Methamphetamine was the most commonly encountered substance in these blood cases as it was found in four cases with a mean concentration of 0.35mg/L (range: 0.16 – 0.66mg/L). Amphetamine was detected in two blood specimens (0.01 and 0.04mg/L) as was THC-COOH (6 and 86ng/mL). Finally, THC was found in one of these blood specimens at a concentration of 8ng/mL.

The 31 cases that had both blood and urine specimens associated with them pertained to 26 females and five males whose average age was 29 years (range: 17 – 57 years). Of these 31 cases, seven had no drugs detected in either blood or urine. Ten blood specimens were positive for ethanol at a mean concentration of 0.11% (w/v) with a range of 0.02 – 0.37% (w/v). THC and THC-COOH were the most commonly encountered substances in these blood specimens found in six and four cases, respectively. THC mean concentration was 2ng/mL with a range of 1 – 4ng/mL while THC-COOH mean concentration was 20ng/mL with a range of 6 – 59 ng/mL. Other psychoactive compounds were also detected in these blood specimens as presented in Table 2. Of the corresponding 31 urine specimens, nine were positive for ethanol at a mean concentration of 0.17% (w/v) with a range of 0.02 – 0.38% (w/v). Methamphetamine, amphetamine, diphenhydramine, THC-COOH, and cocaine were among the most frequently detected drugs in this set of urine specimens (Table 2).

Table 2: Drugs in Cases with both Blood and Urine Specimens

Compound	Frequency in Blood	Frequency in Urine
Benzoylecgonine	3	4
Methamphetamine	3	6
Amphetamine	2	6
Cocaethylene	2	4
Methadone	2	3
Diphenhydramine	2	5
Cocaine	1	4
Citalopram	1	0
Chlordiazepoxide	1	0
Nordiazepam	1	0
Oxycodone	1	3
Trazodone	1	1
Venlafaxine	1	1
Levamisole	0	3
THC-COOH	0	4
Paroxetine	0	1
Hydroxyzine	0	1
Dextromethorphan	0	1

This study demonstrates the variety of substances that are commonly encountered in alleged DFSA victims' toxicology specimens. Ethanol, cocaine, methamphetamine, cannabis, and diphenhydramine are among the most frequently encountered drugs in DFSA case investigations. Interestingly, 44% of the cases reported early enough for a blood collection to take place pertained to male victims, suggesting males are more likely to report early on to the authorities that they may be victims of DFSA but females often delay the reporting, thus rendering blood collection useless. This study will improve the ability of forensic toxicologists and law enforcement personnel to better participate in the investigation of such crimes in their own jurisdictions.

DFSA, Toxicology, San Francisco

K63 Epidemiology of Rodenticide Poisoning in Manipal, South India

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After attending this presentation, attendees will gain knowledge about the incidence and prevalence of rodenticide poisoning cases in South India. The toxicoepidemiology of the rodenticide poisoning, will be presented. Attendees will better understand how the hemorrhagic manifestations appear on the body, and the proper diagnosis can be better understood.

This presentation will impact the forensic science community by providing information about hemorrhagic manifestations occurring in rodenticide poisoning and the duration of their occurrence. This research provides more information in an area with little previous research. This presentation will add to research being carried out in forensic medicine by broadening the understanding of how hemorrhagic manifestations occur in rodenticide poisoning cases, enabling a better appreciation of these manifestations in humans.

Human Poisonings due to chemicals like insecticides, rodenticides, etc. commonly occur because of easy accessibility. Poisoning due to rodenticides, even though rare, are not uncommon. The mortality and morbidity due to rodenticides is increasing worldwide. Hence, knowledge about the epidemiology and clinical manifestations of rodenticide poisoning is not only essential for the treating doctor, but also to the forensic pathologist. The hemorrhagic manifestations of rodenticide poisoning can sometimes mimic contusions, posing problems while interpreting the injuries.

In this retrospective research, the toxic epidemiology of fatal poisoning due to rodenticides in this part of the world is described.

The hemorrhagic manifestations and altered laboratory findings in the victims will also be discussed.

In the present study, fatal rodenticide cases constituted 13.89% of the total poisoning cases, with the majority of the victims being male. The age of the victims ranged from 2 to 82 years. External hemorrhages were present in only five cases, although hemorrhage in Gastrointestinal Tract (GIT) was seen in a maximum number of victims. The prothrombin time (PT) was increased in 21 cases. The enzymes such as Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were raised in 20 cases and total bilirubin was raised in 13 cases.

Rodenticides belong to a category of pest control chemicals intended to kill rodents. The chemicals which act as rodenticides are anticoagulants like warfarins, superwarfarines, thallium, phosphorous, metal phosphides, barium carbonate, red squill, strychnine, etc. Though the mechanism of action of various rodenticides is different, all lead to coagulopathy. Various studies done in the past have suggested the hemorrhagic manifestations due to altered coagulation profiles were induced by these rodenticides.[1-3] Substantial ingestion produces epistaxis, gingival bleeding, widespread bruising, hematomas, aematuria with flank pain, menorrhagia, gastrointestinal bleeding, rectal bleeding, and hemorrhage into any internal organ. Spontaneous hemoperitoneum has been described. Severe blood loss may result in hypovolaemic shock, coma, and death.[1] Hematoma and hemarthrosis as reported by Greeff, M.C., *et al*. were observed in children who accidentally consumed redenticide.[4] Cutaneous hemorrhage and hematemesis were also observed by Dolin E *et al*. in their study.[5] Similar observations were made in this study also.

In conclusion, hemorrhagic manifestations as a result of rodenticide poisoning can be misinterpreted as being due to assault. The differential diagnosis is quite broad and includes all causes of vitamin K deficiency, Disseminated Intravascular Coagulation (DIC), and liver disease. Perusal of hospital records is highly recommended for coagulation profile and is of paramount importance while concluding mode, manner, and cause of death. Rodenticide poisoning as a probable cause should be considered in nontraumatic bruises associated with suspected poisoning cases encountered at autopsy.

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Epidemiology, Rodenticide, Hemorrhages

K64 Stability of Seven Benzodiazepines Together With Zolpidem, Methodone, and Propoxyphene in Bloodstains

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After attending this presentation, attendees will learn about another aspect of bloodstain analysis. The goal of this presentation is to emphasize the potential interest in toxicological analysis of bloodstains.

This presentation will impact the forensic science community by highlighting another aspect of bloodstain analysis that should be valued in forensic practice.

Blood ranks among the most usual kind of physical evidence encountered on a crime scene. Individualization of human blood has been performed for decades by using the ABO system and, more recently, DNA typing granted the forensic scientist a high-performance tool for this purpose. Forensic toxicology, however, also followed a continuous progress, currently providing the possibility to detect various drugs in very small blood samples. This purportedly offers the opportunity to assay bloodstains for toxicological analysis, which could be of interest in some situations, e.g., determination of the victim's toxicological status even if no corpse is found at the crime scene, or of the perpetrator's status if he/she bled in the surroundings of the crime scene. Moreover, when DNA typing cannot be compared to a reference (the victim and/or any biological element for DNA comparison was not found), detecting drugs in bloodstains could contribute to the victim's identification. Until now, only a few works have dealt with the detection of drugs in such samples, and the stability of drugs in bloodstains under different storage conditions have never been studied before. The stability of seven benzodiazepines (diazepam, bromazepam, clonazepam, alprazolam, clobazam, tetrazepam, and triazolam) were investigated, together with zolpidem, methadone, and propoxyphene over periods ranging from 24 hr to one month under various environmental conditions (drugs were chosen because of their frequent prescription in France).

Drug-free 50µL blood samples were spiked with an amount of 10ng/ml of each analyte (500pg per blood sample) and deposited on a glass slide. After storage at -20°C, +4°C, and +35°C away from light, at +20°C in daylight, away from light, and in "extreme" conditions (outside the laboratory exposed to daylight, wind, and variable temperature), bloodstains were collected after 24hr, 48hr, 72hr, one week, and one month by scratching and by swabbing. When scratched, the bloodstain was weighed and rehydrated for 45 min in 0.5mL ammonium buffer (pH 9.5). Swabbing was performed with swabs previously moistened with saline. Swabs were placed in 0.5mL ammonium buffer, sonicated 15 min, and stored away from light for 45 min before swabs were removed. Liquid/liquid extraction was performed using methylenechloride, N-Heptane, isopropanol (65:25:10, v/v) with Prazepam as an internal standard. Then toxicological analyses were carried out by Ultra Performance Liquid Chromatography/Liquid Chromatography with Tandem Mass Spectrometry (UPLC/MS/MS) under previously reported conditions.

The validation of the method was performed on dried bloodstains stored away from light at room temperature for six hours spiked with the different analytes. Under these analytical conditions, the method appeared sensitive (0.0005<LOQ<0.005ng/mg), linear (LOQ-10ng/mg), and accurate (CV<20%).

Results showed a good stability of all drugs tested even after one month of storage in each condition, except for clonazepam at -20°C (sometimes undetected), and for all drugs tested at +35°C and in "extreme" condition with sometimes up to 50% loss after one month. By scratching or swabbing, each analyte could be detected, except for clonazepam at -20°C. This study shows an acceptable stability of most benzodiazepines, zolpidem, methadone, and dextropropoxyphene in dried bloodstains. It opens the way to a new analytical approach which may enhance the bloodstain pattern analysis of a crime scene.

Bloodstain, Drugs, Stability

K65 Determining Zolpidem Compliance: Urinary Metabolite Detection and Prevalence in Chronic Pain Patients

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After attending this presentation, attendees will be able to describe a Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) method for the simultaneous identification and quantification of zolpidem (Ambien®) and its primary urinary metabolite, zolpidem 4-phenyl carboxylic acid (ZCA), in human urine.

This presentation will impact the forensic science community by offering a novel analytical method for sensitive and specific simultaneous quantification of zolpidem parent and metabolite in a single urine extract, as well as providing useful data detailing zolpidem metabolite prevalence in a chronic pain patient population.

Introduction: Zolpidem is the most prescribed insomnia treatment in the United States; however, little is known about zolpidem metabolite excretion in chronic pain patients. As zolpidem is extensively metabolized *in vivo*, metabolite detection may provide improved accuracy for compliance determinations, thereby improving clinical decisions and treatment courses. It is believed that no reported method simultaneously quantifies both parent zolpidem and ZCA in urine.

Method: This study was IRB-approved. Zolpidem and ZCA were extracted from 1mL human urine by mixed-mode solid-phase extraction following buffering with 0.1M acetic acid. Samples were eluted, evaporated to dryness, and reconstituted in 200µL aqueous mobile phase. Samples were injected onto an LC/MS/MS instrument comprised of a Shimadzu Prominence HPLC and ABSciex™ API 3200 tandem mass spectrometer. Ionization was by electrospray (positive mode) with Multiple Reaction Monitoring (MRM) mode employed for detection and quantification. Gradient chromatographic separation starting at 20% B (0.1% formic acid in acetonitrile) was achieved using a C_{18} column (100 x 2.1mm, 3µm particle). Flow rate was 0.7mL/min with an overall run time of 1.8 min.

Results: Conservative Limits Of Quantification (LOQ) were 4ng/mL for both analytes. The assay was validated for linearity from 4 – 1,000ng/mL for zolpidem and 4 – 5,000ng/mL for ZCA (r^2>0.990 and concentrations within ±15% of target). Inter-day recovery (bias) and imprecision (n=20) were 100% – 107% of target and 2.4% – 3.7% relative standard deviation, respectively. Extraction efficiencies were 78% – 90%. Freeze-thaw, processed sample, and autosampler stability were examined (n=6 each), with concentration changes <6.0% observed in all cases. No quantifiable carryover was observed at the method Upper Limit Of Quantification (ULOQ).

A total of 3,264 urine samples were obtained from chronic pain patients over five months and analyzed, with 3,142 (96.3%) meeting qualitative acceptance criteria. Results were de-identified and examined for zolpidem and ZCA prevalence, with concentrations normalized to urine-specific gravity. Zolpidem was detected > LOQ in 720 specimens (22.9%) while ZCA was detected in 1,579 specimens (50.3%). Two specimens (0.06%) contained zolpidem > ULOQ and 45 specimens (1.43%) contained ZCA > ULOQ. Of specimens within the dynamic linear range, median (range) zolpidem and ZCA concentrations were 28.3 (4.08 – 805) ng/mL and 2,038 (4.53 – 23,000) ng/mL, respectively. Only five specimens (0.16%) contained zolpidem alone (median concentration 488ng/mL). As ZCA was observed without parent zolpidem in 864 samples, addition of this metabolite to the assay increased detection rates by 27.5% in this cohort.

Conclusions: An LC/MS/MS method for simultaneous detection and quantification of zolpidem and ZCA in human urine is presented. Addition of zolpidem metabolite to compliance determinations resulted in substantially more positive samples compared to zolpidem alone at the same LOQ. This method is rapid and conducive to a high-throughput environment. Improved detection windows for zolpidem intake should prove useful in both clinical and forensic settings.

Zolpidem Metabolite, Compliance, LC/MS/MS

K66 Detection of Volatiles in Postmortem Samples by Headspace Gas Chromatography With Mass Spectrometry

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After attending this presentation, attendees will become knowledgeable about the detection of toxic volatiles in postmortem specimens such as blood, lung, and brain using Headspace/Gas Chromatography with Mass Spectrometry (HS/GC/MS). Four cases of death caused by toluene, difluoroethane, difluorochloromethane, and nitromethane abuse will be discussed.

This presentation will impact the forensic science community by providing a more reliable method for the detection of volatiles in postmortem specimens.

Volatile inhalants have become a common and dangerous substance of abuse. These inhalants include a broad range of chemicals such as volatile organic solvents, aerosol propellants, and other gases which are readily available, easily purchased, and can be used without supervision or accessories. The mechanism of acute intoxication and death due to inhalant abuse is through fatal ventricular arrhythmia, asphyxiation, and pulmonary edema.

Undoubtedly, the ability to confirm these gases in autopsy specimens presents unique challenges to the forensic toxicologist. Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID) is currently the most widely applied technique in determining the presence of volatile compounds. However, this method does not provide sufficient data for identifying poorly combustible gases. In these cases, HS/GC/MS is more advantageous because it provides spectral data specific to the volatile compound that can be matched with data stored in the NIST library for identification and confirmation. Postmortem samples such as blood, brain, and lung are collected by the medical examiners in a headspace vial and analyzed by HS/GC/MS. The analysis of these cases was performed using an Agilent 7890 GC equipped with Agilent 5975 inert, triple axis Mass Selective Detector (MSD) utilizing a split injection of 100:1. The column was DB-VRX (40m x 0.180mm x 0.100µm) and helium was used as the carrier gas. The oven was programmed for an initial temperature of 55°C that ramped to a final 80°C at a rate of 20°C/min and the entire run time was 11.25 min.

Case 1: Presents a 58-year-old White male found dead with evidence of spray paint on the fingers and face; spray paint cans were located at scene. Upon analysis, toluene was found in his blood sample. Toluene, or methylbenzene, is used in the production of benzene, solvent-based cleaning agents, household aerosols, nail polish, paints and paint thinners, lacquers, adhesives, and as a gasoline additive.

Case 2: Presents a 50-year-old female found with a spray can of Dust Off™ in her hand. Dust Off™, the main ingredient being difluoroethane, is becoming increasing favorable to inhale.

Case 3: Presents a 39-year-old White male found dead, lying prone beside an air conditioning unit, with his mouth against a pipe connected to the unit. Diflurochloromethane, also known as Freon, is growing in popularity because of its ease in being inhaled from outdoor units.

Case 4: Presents a 56-year-old White male that involves nitromethane, which is a component of airplane fluid. In this unique case, the decedent died as a result of consuming airplane fluid in an attempt to get drunk.

Volatiles, Headspace Gas Chromatography, Postmortem

K67 Fatal Intravenous Injection of Oral Therapeutic Drugs in an Elderly Patient

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After attending this presentation, attendees will be more conscious of the potential lethal effects following adverse reactions due to erroneous intravenous administration of oral therapeutic drugs.

This presentation will impact the forensic science community by promoting awareness of rare lethal therapeutic errors.

A 77-year-old male resident in a retirement home, suffering cardiac failure, severe neurological diseases, and dysphagia, died immediately after intravenous administration of a mixed compound, obtained by manual fragmentation of one tablet Respicur® 200mg (theophylline), one tablet of Dintoina® 100mg (phenytoin sodium salt), one tablet Luminale® 100mg (phenobarbital sodium), and a few milliliters of water. The nurse, educated in an Eastern European country and recently employed in Italy, intentionally injected the compound into a jugular catheter to bypass daily difficulties in oral administration, since the patient's parents had refused gastrostomy. A few minutes after the drug-blend injection, the patient showed convulsions, loss of consciousness with cardiac arrest.

Autopsy showed myocardiosclerosis and previous myocardial infarction, pulmonary emphysema, exogenous lipid pneumonia, interstitial fibrosis due to previous repeated gastric aspirations, remarkable congestion in the residual alveolar septa, and no emboli in the pulmonary vessels.

Toxicological analyses on the jugular catheter and syringe revealed extremely high concentrations of all three drugs, as expected from the unusual administration procedure. Toxicological analyses on biological specimens showed drug levels below the maximum therapeutic concentration (left ventricular blood: phenobarbital 15.33mcg/mL; theophylline 1.97mcg/mL; phenytoin 3.70mcg/mL; right succlavian artery blood: phenobarbital, 18.60mcg/mL; theophylline, 2.92mcg/mL; phenytoin, 5.26mcg/mL; left jugular cath residual blood: phenobarbital, 492.3mcg/mL; theophylline, 1395mcg/mL; phenytoin, 463.3mcg/mL). In fact, some degree of postmortem redistribution is expected to have occurred, considering the autopsy was performed three days after death.

Death was caused in this elderly patient by acute phenobarbital, phenytoin, and theophylline toxicity, following erroneous intravenous administration of oral therapeutic doses.

Severe theophylline-related arrhythmias happened very quickly after inoculation of the drug-blend, since the immediate and complete bioavailability produced extremely high concentrations, incomparable to any model of toxic overdose. The total lack of drug metabolism due to first hepatic passage was also responsible for this huge concentration. Moreover, intravenous injection at the left jugular site realized an exceptional condition inoculating theophylline very close to the heart, target of toxicity, and obtaining a sort of topic toxic effect at dramatically high concentrations.

The unusual way of administration also suggests phenytoin-related arrhythmias, as discussed for theophilline. Phenytoin is an anticonvulsant drug usually administered in tablets for chronic therapy, and intravenously at higher dosages for the treatment of acute epileptic seizures. In these cases, rapid parenteral injection can induce cardiac arrhythmias, while oral overdoses usually produce only neurological toxic effects.

Theophylline dose-related toxicity on the central nervous system should also be considered in the mechanism of lethality. On the contrary, the role of phenobarbital neurotoxicity is ruled out, since death rapidly took place and this barbiturate needs a longer time span to cross the hematoencephalic barrier.

Other possible lethal effects related to chemico-physical properties of excipients contained in the micro-fragments of injected tablet-mixture have been considered, but no relevant toxicological or hystopathological findings were noted.

In conclusion, this is the first case in the forensic literature reporting fatality by erroneous parenteral administration of oral therapeutic drugs. This study also points out that in similar occurrences postmortem analytical data have limited value. For this reason, the forensic scientist takes advantage in the diagnosis by the application of uses an appropriate methodology evaluating a complex of elements (circumstantial data; pathology; analytical data), but mainly inquiring/discussing evaluating the pharmacodynamic of each drug in relation to this bizarre irregular method of administration.

Teophylline Toxicity, Phenytoin Toxicity, Therapeutic Error

K68 Postmortem Redistribution and Necrokinetics of Amphetamine, Cocaine, Morphine, and Oxycodone During Post-Embalming Decomposition

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WITHDRAWN

K69 Cannabinoids in 105 Postmortem Forensic Toxicology Cases

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After attending this presentation, attendees will understand the usefulness and value of postmortem cannabinoid analysis and will have a better understanding of the potential cardiotoxic effects of these compounds in humans.

This presentation will impact the forensic science community by providing postmortem cannabinoid incidence data among different types of deaths and by offering postmortem cannabinoid blood reference concentrations.

Between July 1, 2010, and June 30, 2011, the laboratory performed 1,338 postmortem toxicologic evaluations. Cannabinoids were confirmed/quantified in 105 cases (7.8%) comprising of 31 naturals, 31 homicides, 30 accidents, 12 suicides, and one undetermined. Decedents averaged 40 years (range: 16 – 71) and were predominantly male (72%).

Of the 31 naturals, one had antemortem whole blood (AMB) where only THC-COOH was found (11ng/mL). Twenty-eight had peripheral blood (BLP) where THC (n=25; mean: 8; range 1 – 48ng/mL), THC-COOH (n=22; mean: 75; range 5 – 640ng/mL), and THC-OH (n-=4; mean: 77; range: 17 – 210ng/mL) were found. One natural had central/cardiac blood (BLC) and only THC-COOH was detected (69ng/nL). The final natural case involved a decomposed decedent with no blood but THC-COOH was measured in her liver (2.6mg/kg).

In the 31 homicides, four cases had AMB and THC (n=3; mean: 2; range 1 – 5ng/mL), THC-COOH (n=4; mean: 31; range 10 – 78ng/mL), and THC-OH (n=1; 6ng/mL) were detected. Seventeen had BLP and THC (n=15; mean: 21; range 1 – 120ng/mL), THC-

COOH (n=15; mean: 39; range: 1 – 170ng/mL), and THC-OH (n=2; 6 and 14ng/mL) were detected. Nine of the homicides had BLC and THC (n=5; mean: 7; range: 1 – 21ng/nL), THC-COOH (n=9; mean: 24; range: 7 – 96ng/mL), and THC-OH (n=1; 6ng/mL) were detected. The final homicide involved a decomposed decedent with no blood but THC-COOH was measured in her liver at a concentration of 1.4mg/kg.

In the 30 accidents, BLP and BLC existed in 27 and 3 cases, respectively. THC (n=20; mean: 6; range: 1 – 27ng/mL), THC-COOH (n=25; mean: 35; range: 5 – 330ng/mL), and THC-OH (n=1; 5ng/mL) were detected. In cases with BLC, THC (n=1; 3ng/mL) and THC-COOH (n=3; mean: 39; range: 5 – 89ng/mL) were found.

In the 12 suicides, only THC (n=8; mean: 7; range 2 – 24ng/mL) and THC-COOH (n=9; mean: 23; range 6 – 51ng/mL) were detected. Finally, in the one undetermined case, THC and THC-COOH were measured in BLP at 6 and 13ng/mL, respectively.

Comparison of postmortem BLP cannabinoid concentrations among types of cases suggest that THC-COOH averages the highest in natural deaths (75ng/mL, which is more than double its concentration in any other manner of death) whereas THC concentrations run on average three times higher in homicides (21ng/mL) than in any other manner of death. THC-OH, most often found when cannabis-containing products are eaten, averaged 77ng/mL in natural deaths as compared to only 10 and 5ng/mL in homicides and accidents, respectively.

Cannabinoids were not listed in the Cause of Death (COD) in any of the 31 homicides or in any of the 31 natural deaths, but featured as a Significant Other Condition (SOC) in 12 of the homicides (39%) and in 23 of the naturals (74%). Cannabinoids were listed in the COD in 8 of the 30 accidents (27%) and listed as an SOC in an additional 12 of these cases (40%). Cannabinoids were listed in the COD in one of the 12 suicides (8%) and were listed as an SOC in five more suicides (42%). Finally, the one undetermined death listed cannabinoids in the COD together with morphine.

Closer examination of the 31 natural deaths suggests that 84% of these (n=26) showed significant cardiac pathology such as hypertensive heart disease, atherosclerotic cardiovascular disease, and cardiomegaly. In three of these cases, the medical examiner was of the opinion that the decedent had suffered probable lethal cardiac arrhythmia which has previously been reported in the clinical literature as a possible toxic manifestation of cannabis overdose.

This study is the first of its kind and demonstrates the usefulness of cannabinoid analyses as part of every death investigation and provides postmortem concentration reference data that will improve the ability of toxicologists, medical examiners, coroners, and others to evaluate cannabinoid concentrations in human postmortem specimens as well as their possible contribution to death.

Cannabinoids, Forensic Toxicology, Cardiotoxicity

K70 Tapentadol in Postmortem Casework

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After attending this presentation, attendees will be able to detail the types of postmortem casework associated with tapentadol at various concentrations.

This presentation will impact the forensic science community by providing information regarding tapentadol as it relates to cause and manner of death determinations.

Tapentadol (Nucynta®, Palexia®, Zyntap®) is a mu-opioid receptor agonist and norepinephrine reuptake inhibitor. Approved by the Food and Drug Administration in 2009, tapentadol is available as immediate-release tablets of 50, 75, and 100mg and is indicated for acute moderate to severe pain. Adverse reactions associated with tapentadol are generally related to CNS depression including drowsiness, dizziness, headaches, as well as nausea and vomiting. Limited information has been published on tapentadol toxicity. A literature review indicates there are presently only four reported deaths in which the causative agent(s) included tapentadol.

At the North Carolina Office of the Chief Medical Examiner, cases suspicious for toxicological cause or with essentially negative autopsy findings are routinely screened for common over-the-counter, prescription, and illegal drugs via various laboratory techniques. This presentation will detail a group of 12 cases were tapentadol was detected during routine postmortem drug screening in support of cause and manner of death determination. Tapentadol is easily detected by the laboratory's basic organics screen which utilizes both Gas Chromatography with Nitrogen Phosphorus Detection (GC/NPD) and Gas Chromatography Mass Spectrometry (GC/MS). The extraction procedure has been previously described.[1]

Quantification of tapentadol is accomplished using GC-NPD with a calibration curve (blood matrix) and matrix matched positive/negative controls using the extraction procedure referenced above. Linearity, LOD, and LOQ are 0.2-2, 0.025 and 0.2mg/L, respectively. Accuracy and precision in blood (liver) are 99.4 (106) and 4.5 (12)%, respectively. Decedents were divided into groups according to manner of death for the purposes of studying tapentadol concentrations in overdose and non-overdose situations. The accidental and suicidal overdoses were subsequently divided into subgroups for further study: those where tapentadol was determined to contribute to the cause of death (attributed) and those where it was not (unattributed). The deaths in which tapentadol was determined to contribute to the cause of death were further divided into those where tapentadol additively combined with other drugs to cause the death and those where the drug was present in sufficient amounts to have caused the death regardless of other drugs and their concentrations.

Discussion: In all, since December 2010, there have been 12 cases where tapentadol was detected during routine drug screening. Eight cases had paired central and peripheral blood specimens and central/peripheral ratios averaged 1.65 and ranged from 0.54 – 3.3. The mean (median) concentration of tapentadol in central blood was 3.8 (3.3) and concentrations ranged from to <0.2 – 10mg/L. Likewise, for peripheral blood the mean (median) are 2 (2.5) and range is <0.2 – 3.1. For liver, the mean (median) and range are 10 (7.1) and <1 – 25mg/Kg, respectively. Co-intoxicants included antidepressants, antipsychotics, antihistamines, ethanol, cocaine, and miscellaneous CNS depressants.

In conclusion, of the 12 cases studied: 2 (16%) tapentadol were ruled not contributory to death, 7 (58%) were ruled accidental multiple drug intoxication, and 3 (25%) were ruled suicidal multiple drug intoxication. Concentrations of tapentadol in these groups were <0.2, 0.58 – 3.1, and 2.5 – 5.2mg/L, respectively.

Reference:

1. Winecker RE: *Quantification of Antidepressants using Gas Chromatography Mass Spectrometry*; and, *Clinical Applications of Mass Spectrometry*, Hammet-Stabler CH and Garg U, eds. Humana Press, Clifton, NJ. 2010. (pp. 45-56).

Tapentadol, Death Investigation, Toxicology

K71 I'll Huff and I'll Puff: Dust Off™ Canister Abuse

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After attending this presentation, attendees will be aware of the potential for abuse of Dust Off™ in combination with other drugs and the challenges of potentially misleading crime scene evidence.

This presentation will impact the forensic science community by illustrating how simple household products can be abused in conjunction with other drugs.

This presentation discusses a number of inhalant deaths that have occurred in Miami-Dade and Collier County, Florida, since 2010.

The Medical Examiner's Department has investigated six cases during this time period in which the decedents were engaged in inhaling, or "huffing," 1,1-difluoroethane found in commercial products.

In addition to 1, 1-difluoroethane, the cases presented included other drugs such as benzodiazepines, opiates, anti-depressants, diphenhydramine, or synthetic cathinones and tryptamines.

The decedents were mostly Caucasian, three males and three females, ranging in age from 26 – 37 years. In all cases, the decedents were declared dead at the scene with no resuscitative efforts employed. All decedents were surrounded by multiple canisters of products containing 1,1-difluoroethane. The propellant, 1, 1-difluoroethane, typically found in products such as Dust Off™, is readily absorbed by the lungs when inhaled, and causes alcohol-like intoxication including drowsiness, lightheadedness, and loss of inhibition. At toxic levels, effects can include asphyxiation and cardiac arrhythmias. Typical users engage in the inhaling of these vapors by expelling the aerosol into a bag that is held over the head or mouth. Death can occur from either an acute episode or chronic abuse of these inhalants. The manufacturer now includes a bitterant agent in their products to deter or discourage huffing practices.

Blood samples collected during the autopsy that were used for the analysis of 1, 1-difluoroethane were stored in glass screw-top tubes. Initial volatile screening for ethanol, methanol, acetone, and isopropanol by headspace gas chromatography indicated an unknown peak on the chromatograms later identified as 1,1-difluoroethane. Follow-up confirmation was performed using solid phase micro-extraction followed by Gas Chromatography Mass Spectrometry (GC/MS). A commercial Dust Off™ product was used as reference standard for the identification of 1,1-difluoroethane. A working stock solution was prepared by spraying the aerosol into a headspace vial and capping immediately. A working control was prepared by taking a 100µL aliquot of the headspace with a gas-tight syringe from the working stock solution and infusing it into a sealed headspace vial containing internal standard (n-propanol, 15mg/L in de-ionized water). Samples were prepared by adding 1mL of blood to a vial containing 1mL of internal standard. All samples and controls were heated at 65°C for a period of 15 min prior to analysis. The analysis was performed by solid phase micro-extraction using a 75µm Carboxen-PDMS fiber (Supelco, Inc). The fiber was exposed to the samples and controls for 5 min prior to injection into the GC/MS. A 60m x 0.25mm I.D. x 1.4µm Rtx-VMS column (Restek, Inc.) was employed. Analysis was performed in the full scan electron ionization mode, with identification based on spectral library matching. The primary ions used for identification are m/z 51 (base peak), m/z 65 (major ion), and m/z 47 (major ion).

Despite the presence of multiple canisters at each scene, the cause of death is still in question. Toxicology findings in these cases indicated the decedents were abusing more than just Dust Off™. Initial interpretations based on the scene alone could be misleading without extensive toxicology follow-up. In only one of the presented cases was the cause of death solely attributed to 1,1-difluoroethane toxicity. The remaining cases are still pending the pathologist's findings due to the presence of other drugs.

Case	1, 1-difluoroethane	Other Drugs Present	Date
1	Detected	Buprenorphine, Norbuprenorphine, Diazepam, Nordiazepam, Temazepam	July 2010
2	Detected	Diphenhydramine, Clomipramine, Norclomipramine	October 2010
3	Detected	Alprazolam, Citalopram, Norcitalopram, Diphenhydramine	May 2011
4	Detected	MDPV, MDMA, BZP, TFMPP, 5-MeO-DiPT, Dextromethorphan	November 2011
5	Detected	Diphenhydramine	September 2011
6	Detected	Alprazolam, Oxycodone, Tramadol	February 2012

Inhalant, 1, 1-Difluorethane, SPME

K72 Scientific Method for Controlled Substance Analog Determination

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After attending this presentation, attendees will understand relevant scientific concepts needed to comprehensively evaluate non-controlled substances as potential analogs of controlled substances, acceptance criteria associated with those concepts, the ability to establish laboratory practices to present scientific data regarding analog determination in court, and assist in the scientific prosecution or defense of analog drug cases.

This presentation will impact the forensic science community by providing laboratories with objective, science-based criteria to evaluate compounds and a means to establish consistency in analog determinations made in laboratories across the country. This presentation will introduce toxicological, chemical, and synthetic concepts surrounding the evaluation of potential controlled substance analogs and offer a scientific method for this evaluation.

The Advisory Committee for the Evaluation of Controlled Substance Analogs (ACECSA) was established by scientists from federal, state and private forensic laboratories, academia, and law to develop a scientifically valid and peer-reviewed means of evaluating the analog status of non-controlled substances and serve as a resource to law enforcement, legal counsel, laboratories, and government agencies in the scientific categorization of non-controlled substances. The Committee was gathered intentionally to maintain an independent, un-biased, and un-weighted stance in the scientific and legal communities. The main goal of constructing this group was to address a lack within the forensic chemistry field regarding the evaluation of analogs. To date, there are no guidelines, recommendations, or methods that exist in our field and no consensus or consistency in the determination of these compounds. Scientifically-sound guidelines or recommendations for analog determination are needed in the forensic arena in response to the overwhelming "designer drug" explosion and the difficult task of legislating potentially harmful new drugs.

The members of the ACECSA, in collaboration with national and international subject-matter experts, developed five aspects of a compound that should be included in evaluating analog status: Chemical Structure; Physicochemical Properties; QSAR/Computational Chemistry; Synthetic Pathway; and, Toxicology/Pharmacology will present applicable concepts and associated acceptance criteria to demonstrate the comprehensive approach to analog determination.

The Chemical Structure subcommittee aims to develop a process by which potential controlled substance analogs are evaluated and compared on the basis of their structural similarity. This structure evaluation process focuses on both 2D and 3D aspects of a chemical's structure. Initial investigations look at core structures and functional groups.

The use of physicochemical properties in proposing potential new drug candidates has its basis primarily in the bioavailability of the compound *in vivo*. For example, solubility, partition coefficient, and pKa/pKb provide preliminary *in vitro* guidance as to the potential bioavailability.

Solubility primarily affects the dosage form and route of administration. Partition coefficient is used to predict membrane permeability. The compound's acidity or basicity determines where an orally-administered drug might be absorbed in the body. These properties will be retrieved from scientific literature (if available) or

may be calculated by modern computer programs designed and used for this purpose.

The Quantitative Structure-Activity Relationship/Computational Chemistry subcommittee will utilize available predicted activities for new chemicals. It will also evaluate the utility of similarity co-efficient models such as Tanimoto.

The goal of the Synthetic Pathway subcommittee is to analyze the structure of potential controlled substance analogs by the pathway in which they were created, i.e., deducing their chemical construction. The pathway of chemical synthesis of any organic compound can be modified by employing different building blocks; this serves as a rapid means to generate analogs of beneficial, or controlled, chemical compounds.

The Toxicology subcommittee will evaluate available pharmacological and toxicological data regarding novel compounds and compare their properties to existing controlled or scheduled drugs. This will include receptor binding and functional assay data, human and animal dosing studies, case reports, behavioral studies, adverse event reporting, and epidemiological data with clinical indicators, provided in the latter two cases that they are accompanied by analytical confirmation of the substance identity.

Analog, Controlled Substance, Method of Evaluation

K73 Cross-Reactivity of Cathinone Derivatives and Other Designer Drugs in Commercial Enzyme-Linked Immunosorbent Assays

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After attending this presentation, attendees will gain an understanding of designer drugs, particularly "bath salts" or cathinone derivatives, and their prevalence in our society. In addition, the audience will also learn about the immunoassay techniques involved in screening for these substances and how the compounds cross-react in such commercial assays.

This presentation will impact the forensic science community by serving as a resource for cross-reactivity data of designer drugs in commercial Enzyme-Linked Immunosorbent Assays (ELISAs), an important factor to consider when screening biological specimens for drugs of abuse.

Designer drugs have been no stranger to the drug market in the United States over the past few decades. Recently, "legal highs" in the form of "bath salts" or "research chemicals" have dominated the drug scene as substances that are labeled "not for human consumption" in order to bypass recent regulations. While a number of bans have been put in place regarding such compounds, the abuse of these designer drugs has been on the rise while manufacturers have been staying one step ahead of the law with constantly evolving modifications to structures. When an intoxication or fatality occurs, presumptive techniques, such as immunoassays, are employed to quickly screen biological specimens for common drugs of abuse. However, since cathinone derivatives are fairly new, few assays have been created for the detection of such compounds. It is hypothesized that during routine drug screens by ELISA, the cathinone derivatives and other designer drugs may be missed. In a toxicology lab, a negative screen would not be further investigated and the substances may never be detected. For this reason, it is important to investigate the cross-reactivity of such designer drugs by analyzing across several commercial immunoassays.

In this large-scale experiment, ELISA reagents from Immunalysis, Neogen®, OraSure®, and Randox® were evaluated to determine the cross-reactivity of 30 designer drugs, including 24 phenylethylamines (including MDPV and eight cathinone derivatives), 3 piperazines, and 3 tryptamines. The study determined the percent cross-reactivity for the compounds in 16 commercial immunoassays, targeting amphetamine, methamphetamine/MDMA, benzylpiperazine, mephentermine, methylphenidate, ketamine, MDPV, mephedrone/methcathinone, PCP, and cotinine.

Cross-reactivity towards the "bath salts" was 0.5% – 4% in the assays targeting other phenylethylamines such as amphetamine or methamphetamine/MDMA. Compounds such as MDA, MDMA, ethylamphetamine, and α-methyltryptamine (AMT) demonstrated cross-reactivities in the range of 30% – 250%, but were consistent with both the manufacturer's inserts and published literature. Some assays, such as BZP, cotinine, PCP, mephentermine, methylphenidate, ketamine, and MDPV demonstrated almost no cross-reactivity toward any of the analytes evaluated. The mephedrone/methcathinone kit from Randox® demonstrated cross-reactivity toward cathinone derivatives—with false positives occurring at concentrations as low as 150ng/mL. The mephedrone/methcathinone kit was not a suitable assay for detecting other more traditional amphetamine-derived compounds but may be more fitting for screening postmortem specimens for "bath salts" when putrefactive amines may be present.

This comprehensive study determined the cross-reactivity for 30 designer drugs in biological specimens across 16 commercial immunoassay reagents. Very few "false positives" were observed in this study, which indicates the selectivity of the immunoassays and the antibodies that are employed. However, the fact that very few additional compounds were detected demonstrates a need for more broad-range screening techniques to be applied when analyzing biological specimens by immunoassays for drugs of abuse, specifically the more recent designer drugs.

Immunoassay, Designer Drugs, Cross-Reactivity

K74 Analysis of Synthetic Cannabinoids Using Disposable Micropipette Extraction Tips and LC/MS

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After attending this presentation, attendees will be able to evaluate the effectiveness of disposable micropipette tips for the extraction of synthetic cannabinoids from biological samples, relative to traditional liquid/liquid extraction.

This presentation will impact the forensic science community by describing a rapid drug screening technique for a class of drugs that is becoming increasingly popular. This technique can aid in the regulation of these "legal high" products and identify their role in criminal activity or death investigations.

This project was designed to evaluate the applicability of the use of solid phase micropipette extraction tips for the isolation of synthetic cannabinoids from biological samples.

Synthetic cannabinoids are a rapidly growing class of drugs that have similar effects to those of marijuana. The scope of this class of drugs is fast-growing because their structures are easily manipulated, but can still produce cannabis-like effects. The chemical constituents of these synthetic marijuana products change frequently as attempts to regulate them evolve. Being able to detect these various drugs in biological samples has significant forensic toxicology applications. Previously, liquid/liquid extraction procedures have been performed to isolate the drugs prior to analysis by Liquid Chromatography Tandem Mass Spectrometric (LC/MS/MS) analysis; however, liquid/liquid extractions can be very time consuming, generate large amounts of waste, and involve multiple manipulations, resulting in reduced recovery.

This study describes the development of a rapid method to screen for the model synthetic cannabinoids AM-1248, AM-2201,

JWH-122, JWH-210, and XLR-11using disposable micropipette extraction tips. Disposable micropipette extraction is a novel technique based on solid phase extraction. The pipette tips contain a sorbent material that binds to the sample as the solution is aspirated and expelled through the frit in the tip. These tips are advantageous over traditional liquid/liquid extraction because they are more efficient, rapid, and require lower solvent volume. Reduced solvent waste is an environmental benefit of this approach. Once extractions are performed, samples are analyzed LC/MS/MS.

Variables such as time for extraction, total solvent volume, and sample volume were evaluated as part of this assessment. A previously reported liquid/liquid extraction method was evaluated for comparison to the proposed extractions with disposable micropipette tips. The liquid/liquid extraction is performed using 1mL of sample which is acidified and extracted with chloroform/isopropanol/n-heptane, 50/17/33. Liquid/liquid extractions from serum demonstrated R^2 values above 0.98 with a linear range of 0.1 – 15 ng/mL.

Preliminary results show that the individual 10 ng/mL standards can be extracted from the micropipette tips with a significant increase in abundance when compared to liquid/liquid extractions of the 10ng/mL standards. Abundances for the micropipette tips were almost five times greater than that of liquid/liquid extractions of samples of the same concentration. Extractions with the tips also proved to be less time consuming. To prepare calibrators and perform extractions with the tips takes 2 hr as compared to 4 hr with liquid/liquid extractions. Solvent and sample volume are also decreased when utilizing the tips. The micropipette tips use 2.25mL of solvent and 0.50mL of sample volume while liquid/liquid extractions use 4.20mL of solvent and 1 – 2mL of sample volume.

Based on the work described above, disposable micropipette tips successfully extract standards of AM-1248, AM-2201, JWH-122, JWH-210, and XLR-11. The micropipette tip extraction thus far proves to have higher extraction efficiency while utilizing less sample and solvent volume.

Cannabinoids, Pipette Tip Extracts, Designer Drugs

K75 Cross-Reactivity of Designer Phenethylamines With the Emit® II Plus Amphetamine/Methamphetamine Assay in Urine

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The goal of this presentation is to provide attendees with cross-reactivity information for 4-methylethcathinone and other psychoactive phenethylamines that may aid with screening for designer drugs in postmortem and police cases. The discussion will include the parameters of the analysis as well as some specific case examples.

This presentation will impact the forensic science community by providing a fast means of screening for phenethylamine designer drugs in urine.

4-methylethcathinone (4-MEC) is a designer stimulant that has been a popular drug of abuse in New Zealand. It has been sold in the United States in bath salts products and as a research chemical. It is reportedly abused for psychoactive effects, including euphoria and alertness. 4-MEC was recently indicated in the suicide death of a New Zealand teenager and is an important compound to include in forensic toxicology testing.

NMS Labs recently reported two positive results for 4-MEC in blood that also had positive amphetamine immunoassay results in urine. None of the usual amphetamines, including amphetamine and methamphetamine, were detected in the blood using Liquid Chromatography With Tandem Mass Spectrometry (LC/MS/MS). Since the structure of 4-MEC and other phenethylamines appears similar to amphetamine, it may be possible to use existing immunoassay kits to screen for this class of designer drugs.

There have been several recent reports of designer drugs producing similar positive screening results. Mephedrone was found to cross react with a methamphetamine ELISA in postmortem blood.[1] In addition, Methylenedioxypyrovalerone (MDPV) was found to cross react with PCP.[2]

In order to further characterize these potential false positive results, the cross-reactivities for several popular phenethylamines were determined by spiking standards into blank human urine. Analysis was performed using the Emit® II Plus Monoclonal Amphetamine/Methamphetamine Assay (Syva). Amphetamine, methamphetamine, cathinone, methacathinone, mephedrone, methylone, MDPV, alpha-pyrrolidinovalerophenone (alpha-PVP), 4-MEC, pentedrone, buphedrone, and naphyrone were evaluated between 500 and 10,000 ng/mL. The final concentrations in urine were verified by LC/MS/MS for those compounds with a quantitative method available. The results of this cross-reactivity testing will be presented along with some case examples.

The landscape of the designer drug market has been changing rapidly, making it difficult to develop sensitive methods for detection. Structural similarities may be used as a guide to select existing screening methods that may be sensitive to emerging designer drugs. These findings also indicate that unconfirmed methamphetamine screens could potentially contain phenethylamines. While there may be many explanations for false positive screens, the possibility of bath salt ingestion should be added to the list for consideration by medical and laboratory professionals.

This is especially important considering that one of the most attractive attributes of designer drugs is their invisibility on standard drug tests. Many analytical laboratories have been developing quantitative methods that can confirm designer phenethylamines in a variety of matrices, but targeted screening with these methods is often cost-prohibitive. The Emit® II Plus and other immunoassay tests are readily available and inexpensive tools that can potentially be validated as designer drug screens.

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Immunoassay, Designer Drug, 4-MEC

K76 Validation of Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Synthetic Cannabinoids Metabolites in Urine

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After attending this presentation, attendees will be able to evaluate the use of immunoassay tests for the detection of synthetic cannabinoid metabolites in urine.

This presentation will impact the forensic science community by outlining an approach to method validation for immunoassay, and by providing an assessment of the merits of an immunoassay approach to the detection of these emerging compounds.

The prevalence and popularity of synthetic cannabinoid drugs has created the need for a low-cost option for screening for the presence of these drug metabolites in biological fluids. Liquid Chromatography/Mass Spectrometry (LC/MS/MS), which is in limited

use as a method for screening for these compounds, requires sample extraction, lengthy run times, and is an expensive approach for high-volume screening. It also requires additional development for every new analyte or new metabolite discovered. Enzyme Linked Immunosorbent Assay (ELISA) is in widespread use for screening for many classes of drugs, and the development and evaluation of two ELISA tests to detect synthetic cannabinoids is described. ELISA is a rapid procedure that uses low-cost reagents and can be readily automated, making it an optimal technique for this process.

Following the production of antibodies in a rabbit model, antisera was harvested and antibodies isolated. After evaluating the performance of antibodies from several animals, the optimum antibody was used in a homogeneous ELISA plate immunoassay on a 96-well plate.

Two ELISA assays were developed, targeted to JWH-018 (1-naphthyl-(1-pentylindol-3-yl)methanone) and JWH-250 (2-(2-methoxyphenyl)-1-(1-pentylindol-3-yl)ethanone), respectively. At the time of development, JWH-018 and JWH-250 were the most prevalent compounds on the illicit drug market.

The assays were validated to determine their performance at the defined cut-off of 5ng/mL. The 5ng/mL cut-off was selected based on analysis of incurred authentic positive urine samples and LC/MS/MS analysis of JWH-018 and JWH-250 metabolite concentrations in authentic samples. Intraday and interday precision were evaluated by analyzing calibrators and controls over 10 days with 2 runs per day. Cut-off calibrators were run in duplicate and the mean OD used to establish the cut-off. A negative control, positive control (20ng/mL), and near cut-off concentration control (10ng/mL) were evaluated and performed acceptably for both assays under these conditions.

The assays showed significant cross reactivity with other synthetic cannabinoid standards, and metabolites. Several compounds including JWH-018 4-OH pentyl, JWH-018-5OH pentyl, JWH-081, JWH-081 4-OH pentyl, JWH-081 5-OH pentyl, JWH-122, JWH-122 5-OH pentyl, AM-2201, AM-2201 4-OH pentyl, and others generated positives on the JWH-018 assay at concentrations of less than 20ng/mL. Fewer compounds including JWH-250, JWH-250 4-OH pentyl, JWH-250 5-OH pentyl, and others generated positives on the JWH-250 assay at the same threshold. Common drugs of abuse and therapeutic drugs did not react at elevated concentrations (>10,000ng/mL).

Validation controls (positives and negatives) as determined by LC/MS/MS were presented to the ELISA methods. Subject samples testing positive for JWH-018 (5-hydroxypentyl) metabolite using LC/MS/MS with a cutoff concentration of 0.1ng/mL were assessed using the JWH-018 Direct ELISA kit. There were 61 of 63 LC/MS/MS positive samples which tested positive by ELISA, while all 51 pedigreed negative samples tested negative by ELISA. Thus, the sensitivity, specificity, and accuracy for the JWH-018 Direct ELISA kit were 96%, 100%, and 98%, respectively.

Subject samples testing positive for JWH-250 (4-hydroxypentyl) metabolite using LC/MS/MS with a cutoff concentration of 0.5ng/mL were assessed using the JWH-250 Direct ELISA kit. There were 32 of 33 LC/MS/MS positive samples which tested positive by ELISA, while all 51 pedigreed negative samples tested negative by ELISA. Thus, the sensitivity, specificity, and accuracy for the JWH-250 Direct ELISA kit were 97%, 100%, and 99%, respectively.

These ELISA assays have proven effective, sensitive, and specific for the purposes of screening for many of the currently popular synthetic cannabinoid compounds. Continued vigilance is needed to ensure that the assays will cross react with newly emerging drugs in this class.

ELISA, Cannabinoids, Designer Drugs

K77 Qualitative Analysis of Designer Stimulants and Bath Salts Chemicals in Blood, Serum/Plasma, and Urine by LCTOF

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After attending this presentation, attendees will be able to discuss the use of Liquid Chromatography Time-Of-Flight Mass Spectrometry (LC/TOF/MS) for the screening of designer stimulants and hallucinogens in biological samples and identify considerations that reduce the risk of false positive findings.

This presentation will impact the forensic science community by increasing forensic toxicologists' understanding of the strengths and limitations of LC/TOF analysis and the need for confirmatory testing by independent methods.

LC/TOF is becoming an increasingly popular technique for drug screening in forensic and clinical toxicology laboratories. The technique is based on high-efficiency liquid chromatographic separations, coupled with a detection system that confirms the identity of the analyte by assessing its retention time and accurate mass relative to an analytical standard and its accurate mass. A panel was developed and validated for the detection of 40 analytes, including popular designer drugs such as MDPV, mephedrone, methylone, ethylone, naphyrone, 5-MeODALT, 5-MeO-DIPT, 2C-D, 2C-E, 2C-H, 2C-I, 2C-T-2, 2C-T-7,and others. The compounds were selected based on the fact that they are scheduled either at the state or federal level and are increasingly being found in "Bath Salts" type of products for purposes of abuse.

Samples (0.5mL) were buffered with 0.1M borax buffer followed by liquid-liquid extraction using 70:30 n-butylchloride:ethyl acetate. The acquisition method used a run time of 10 min with a flow of 0.700mL/min. The method used six representative deuterated internal standards to monitor analyte recovery. A target cut-off of 10ng/mL was selected for most compounds in blood and urine. Criteria used to evaluate positivity were retention time, mass accuracy, and percent concentration compared to the cutoff.

Validation of the assay consisted of assessment of precision around the cut-off, stored sample stability (room temperature (light and dark), refrigerated and frozen), carryover, autosampler stability, interference, sensitivity, and specificity.

The validation of this panel yielded an overall sensitivity of 99.7% with a range of 72% to 100% and selectivity of 99.0% with a range of 79% to 100%. Generally, analyte stability was shown to be sufficient in refrigerated and frozen samples and insufficient otherwise, whether light protected or not. Naphyrone in serum was an exception, which was shown to be unstable after one day. No carryover was detected following a sample spiked at 100 times the cutoff. Potential interferences were investigated by testing various mixed analyte pools, and did not yield any false positive findings.

The method's reproducibility was demonstrated by observing internal standard area, performance of the positive and negative controls, and the limited number of false positives. False positives only appeared to be an issue with isobaric pairs of analytes, such as 3-FMC and flephedrone, and MDMA and mephedrone. For this reason, when either member in the pair is present, confirmation testing will be done for both.

LC/TOF is a useful tool for forensic toxicology screening; however, the limitations of the technique must be acknowledged. Recognizing isobaric compounds is key to using LC/TOF as a screening tool, since the molecular mass of the analytes is the main identifying criteria. The success of the technique is highly demanding of high resolution chromatography and chromatographic quality and stability during the assay. Mass accuracy is one parameter that can help in determining the positivity of an analyte. Ion suppression due to sample matrix is another issue that must be addressed. Using

representative deuterated internal standards is one way of determining the likelihood of ion suppression in a given sample.

LC/TOF, Designer Drugs, Stimulants

K78 Stability of Synthetic Cathinones (Bath Salts) in Toxicology Specimens

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After attending this presentation, attendees will learn about the *in vitro* degradation of synthetic cathinones, often referred to as bath salts. Based on the hypothesis that beta-keto amphetamines are inherently unstable in biological matrices, a preliminary study using several cathinones spiked in blood and urine samples stored at ambient and refrigerated temperatures was evaluated by Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS) and Liquid Chromatography/Time-Of-Flight/Mass Spectrometry (LC/TOF/MS) over a five-week time period. This model system revealed temperature-dependent and compound-specific decay rates among the commonly known cathinones.

This presentation will impact the forensic science community by emphasizing the effects of storage conditions and type of biological sample used to perform analysis. Data reflected a rapid signal degradation, which was accelerated in ambient storage conditions and urine specimens. The values extrapolated from each experiment yielded putative rate constants, which were compared to previous actual cases with confirmed cathinones. The limited data analyzed from the five-week trial with sampling each week produced a broad generalization that could aid interpretation of results, especially when intoxication was observed yet there is an absence of confirmatory signal.

The most stable cathinone in this study was methylenedioxypyrovalerone (MDPV), which retained as much as 29% in blood stored at room temperature. In distinction, naphyrone and mephedrone showed a complete loss of response. Compared to room temperature, refrigerated blood had an overall average increase in stability by 34%. Urine samples at room temperature demonstrated the most dramatic effect between sample and temperature. All cathinones, excluding MDPV, fell sharply at day 8, followed by an even greater drop below 5% or complete loss of signal by day 29. In comparison, refrigerated urine had an overall average increase in stability by 45%. The differences show that biological specimen and temperature significantly affect synthetic cathinone stability. Both specimens produced more stability in refrigerated temperature (4°C) compared to ambient temperature (21°C). The complete or significant loss in signal occurred earliest in urine samples at ambient temperatures.

The lessons learned from this pilot study were applied to actual postmortem and DUI forensic toxicology cases. For postmortem samples collected at autopsy, the urine specimens were stored frozen at -20°C without preservative, while decomposition fluid was stored at 4°C in grey top blood tubes preserved with sodium fluoride. For DUI samples collected, the urine contained no preservative and was refrigerated at 4°C. These specimens were re-analyzed and the rate constant and half-life calculated from the stability experiment were applied and compared to previous cases. The degradation of each cathinone followed a first order rate of decay. MDPV was the compound confirmed in all cases. The rate constant determined from the pilot study agreed fairly well with actual case results.

This study indicates that it is possible for a specimen to generate a false negative result if the specimen was stored at room temperature or analyzed after a significant time delay. Refrigeration proved to lengthen stability for both types of specimens, with refrigerated urine producing the greatest stability. The stability of cathinones in biological samples is extremely important due to its increasing use among drug abusers and lack of experience in toxicology laboratories. Factors that may influence drug stability in stored samples include: storage temperature, storage time, addition of preservatives, and initial condition of the collected sample. Furthermore, LC/TOF/MS analysis revealed that as cathinone levels diminished, their corresponding "reduced" forms became more prevalent. This development may signal the need for reduced derivatives of cathinone standards for use in forensic toxicology confirmations.

Bath Salts, Stability, Toxicology

K79 Forensic Investigation of PSU Herbal Incense Products Using GC/MS and LC/MS/MS

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After attending this presentation, attendees will gain knowledge on herbal incense products as an emerging designer drug, applications of method development for Gas Chromatography-Mass Spectrometry (GC/MS) and Liquid Chromatography with Tandem Mass Spectrometry (LC/MS/MS), in addition to the detection, identification, and quantification of synthetic cannabinoids in two commercially available products, Down2Earth Climax and Wet. In order to aid law enforcement's efforts to contain and prevent the use of these emerging designer drugs by youth, it is essential that the forensic science community develop rapid screening methods for detection that will enable rapid designer drug screening. This research will also provide significant chemical information regarding the unknown compounds in these street samples, and assist with drug awareness in a college town, a prevalent drug-consuming community, by presenting the findings at local high schools and colleges in the area.

This presentation will impact the forensic science community by developing methods for detection that will facilitate rapid designer drug screening, aiding law enforcement's efforts to contain and prevent the use of these emerging drugs.

Legal herbal products, found readily available through the internet and local gift shops, are increasingly being used for recreational drug use by youth. Marketed as herbal incense and specifically labeled "not for human consumption," these products are plant materials sprayed with cannabinoid-related chemicals that users vaporize and inhale.[1,2] Two commercial herbal samples, Wet and Down2Earth Climax, purchased in downtown State College, PA, are investigated and analyzed to determine synthetic cannabinoid presence and quantity. JWH-018, JWH-073, JWH-200, CP-47, 497, and cannabicyclohexanol are analyzed, as these represent five chemicals currently placed on Schedule I classification by the U.S Drug Enforcement Administration (DEA).[2] Scheduling was in response to an increase in the frequency of hospitalizations involving incense inhalation in the United States.[3] In addition to the five Schedule I chemicals listed above, 30 other synthetic cannabinoid related chemicals are being investigated in order to construct a library for instrumental drug screening.

To accomplish synthetic cannabinoid chemical screening, multiple extraction techniques were compared and the QuEChERS (Quick, Easy, Cheap, Rugged, and Safe) extraction method proved to be most suitable.[4] QuEChERS provides a time-effective option by combining the herbal sample with magnesium sulfate and calcium chloride buffering salts and methanol solvent in a 50mL centrifuge tube. After shaking the sample for 5 min, sample centrifuging ultimately allows for removal of the organic phase. Extracts are characterized using a GC/MS and an triple quadrupole LC/MS/MS. Optimized methods, coupled with library construction of synthetic cannabinoid standards, enable simultaneous screening of cannabinoid species, and permit a comparison of the two instrumental setups for drug screening and quantification of street herbal products.

Currently, employee drug testing does not incorporate these compounds, but as more analogs become illegal and available, screening will be forced to expand to include such chemicals. Thus, it is important to develop and validate instrumental methodology for such screening. Traditional crime laboratory drug analyses focus on GC/MS instrumentation, but semi-volatile and thermally unstable compounds may not be suitable. LC/MS/MS methods may prove more suitable for such chemicals. Preliminary results indicate a rapid, effective method for the separation and identification of synthetic cannabinoid standards and commercial herbal samples for GC/MS and LC/MS/MS. Using the method of internal calibration, deuterated analogs are utilized to quantify the synthetic cannabinoids in the samples. Products from the same brand allow for determination of inner-batch variability. Future analyses will expand to incorporate other street samples from the area, and to evaluate sample heterogeneity. Pending method optimization, the research project can be expanded to include other emerging cannabinoid-based drugs that come into vogue.

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Synthetic, Cannabinoids, Screening

K80 Development of an LC/MS/MS Method for 30 Synthetic Cannabinoids and Metabolites

Erica A. Guice, MS, Matt Pentis, BS, Brad T. Holden, BS, and Mounira Ozoor, 1197 Rochester Rd, Suite K, Troy, MI 48083*

After attending this presentation, attendees will: (1) understand the challenges of testing for synthetic cannabinoids; (2) obtain knowledge on the effects of matrix on synthetic cannabinoid testing; and, (3) understand the differences in platform for synthetic cannabinoid testing

This presentation will impact the forensic science community by providing needed information for the detection of several synthetic cannabinoids that are currently being missed by laboratories.

Synthetic cannabinoids have been a topic of much discussion since their popularity as a legal high started in 2004 in Europe. To date, several states have moved to ban the use and sell of synthetic cannabinoids. However, most state and federal law makers have banned specific compounds, not structural moieties, allowing for similar compounds to come onto the market. As such, the detection of synthetic cannabinoids has always been mostly reactive.

Laboratories who generally rely on a screen-confirm methodology for their testing were further hampered until immunoassays for these compounds were developed. And, to date, most of the immunoassays developed are limited and some are already outdated. In order to remain current with the compounds on the market, based on seizures and blogs, Western Slope Laboratory developed a drug-monitoring method using liquid chromatography-online sample extraction-tandem mass spectrometry with full scan orbitrap. This method allows for targeted analysis as well as unknown compound elucidation.

With this methodology, testing is available for 30 synthetic cannabinoid compounds including metabolites. Included in the method are markers for JWH-018, JWH-073, JWH-200, JWH-019, AM-2201, JWH-122, JWH-398, JWH-022, JWH-210, JWH-015, JWH-081, JWH-020, JWH-250, HU-210, AM-694, STS-135, and XLR-11. The method tests for synthetic cannabinoids in urine and saliva.

The method is validated in concentration range of 100pg/mL – 1,000ng/mL with a Lower Limit of Detection (LLOD) below 100pg/mL and a Lower Limit of Quantification (LLOQ) at 100pg/mL. The method is linear in the aforementioned quantification range. The method was tested for matrix suppression and enhancement and none was seen in the quantification window as defined as ±25%. Imprecision has a specification limit of ±20% for all compounds; however, repeated injections (n=10) were under ±10%. Similarly, inaccuracy has a specification limit of ±20%. The method was tested to be accurate at three concentrations (low=250pg/mL, medium=50ng/mL, and high=650ng/mL) for repeated injections (n=10).

Urine samples are hydrolyzed, spiked with internal standards, and injected on the turbulent flow column. Saliva samples are spiked with internal standards, filtered, and injected onto the turbulent flow column. Standards are purchased from Cayman Chemical and Cerilliant. Mobile phase was water and methanol with ammonium formate and ammonium acetate additives. All samples were run on a Transcend TLX-2 (Thermo Scientific) coupled to a Exactive Orbitrap (Thermo Scientific). Run time for the method was under 8 min.

This method is comparable to the quantitative method previously developed at Western Slope Laboratory for synthetic cannabinoid confirmatory services. The drug-monitoring method was able to compare to the confirmatory method for the eight compounds in the confirmatory method; those compounds are JWH-200, JWH-018, JWH-018 N-pentanoic acid, JWH-073, JWH-073 N-Butanoic Acid, AM-2201, AM-694, and HU-210. The quantifiable results were similar (±10%).

In conclusion, a drug-monitoring method was developed to allow for detection of 30 synthetic cannabinoid compounds and metabolites to help in the fight against the use of these compounds. This method allows for both confirmatory testing as well as unknown identification. With this type of methodology, laboratories can now be more proactive.

Synthetic Cannabis, LC/MS/MS, Urine and Saliva

K81 Designer Stimulants and Hallucinogens in Routine Casework

Sherri L. Kacinko, PhD, Wendy R. Adams, PhD, and Barry K. Logan, PhD, NMS Labs, 3701 Welsh Rd, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to discuss the type and frequency of synthetic stimulants and hallucinogens seen in routine casework at a large reference laboratory.

This presentation will impact the forensic science community by providing a comprehensive review of designer drugs being seen in routine postmortem and human performance casework.

Over the past several years, the forensic toxicology community has been challenged with the emergence of large numbers of designer stimulants and hallucinogens in products commonly referred to as "Bath Salts." The speed at which the drug-using community is able to adjust to new legislation and find compounds that are not specifically scheduled has mandated a mechanism to quickly add new compounds to the scope of routine testing. A protocol was developed to rapidly expand the Deconvolution Reporting Software (DRS) library for the Gas Chromatography/Mass Spectrometric (GC/MS) screen used in the laboratory.

Data was extracted from the laboratory information management system for all GC/MS screening analyses from January 1, 2012, to July 26, 2012. All cases in which the presence of at least one

designer drug was indicated were included in the data analysis. A total of 178 such cases were identified. Pentedrone was the most prevalent compound identified (42 cases), followed by alpha-pyrrolidinopentiophenone (Alpha-PVP, 41 cases), dimethylamylamine (DMAA, 36 cases), and methylenedioxypyrovalerone (MDPV, 26 cases). Other drugs identified were methylone (16), 4-methylethcathinone (10), methylbenzylpiperazine (7), pentylone (5), butylone (5), ethylone (4), dimethylamphetamine (4), 3,4-dimethylmethcathinone (3), 1,4-dibenzylpiperazine (3), buphedrone (3), methoxetamine (2), 2C-I (2), and 1 case each of paramethoxymethamphetamine, 2C-H, 5-MeO-DALT, and paramethoxyamphetamine. Twenty-eight cases included multiple compounds. Nine of these contained didesmethylsibutramine, a metabolite of the diet drug sibutramine, but no sibutramine.

The compounds commonly found in "Bath Salts" generally fall into one of several classes of compounds, substituted phenethylamines, beta-keto cathinones, tryptamines, or piperazines. They are abused for their stimulant and/or hallucinogenic properties. The mechanism of action has not been elucidated for each compound. They generally elicit their effects by acting on serotonergic and dopaminergic receptors or by stimulating the production of related neurotransmitters. DMAA has been detected in "Bath Salts" products, specifically "PumpIt Powders," but is not structurally similar to other common bath salt compounds. It is widely available as a supplement and used by body builders and is believed to stimulate the release of catecholamine. It does not work directly at adrenergic receptors. While 92% of cases which had a positive screen for DMAA did not appear to contain any other "Bath Salt," three cases contained DMAA in combination with other compounds. One also contained Alpha-PVP and didesmethylsibutramine, one contained only Alpha-PVP, and one contained methylone.

The review of seven month's worth of routine GC/MS screening data confirms the necessity of establishing a method able to detect designer stimulants and hallucinogens. These compounds are relevant to both postmortem investigations and as substances which can potentially impair driving.

Designer Drugs, Bath Salts, Postmortem

K82 Postmortem Pediatric Toxicology

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After attending this presentation, attendees will gain an appreciation of 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 14th Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings are discussed. As a relative dearth of interpretive information exists involving toxicological findings in the pediatric population, 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 Academy, allows for various perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause-of-death in children.

Dan Isenschmid, PhD, will be presenting a case where a 2-month infant was characterized as being "colicky." The teenage mother, an illegal alien from Mexico, may have seen a doctor in Mexico and received medications for the infant. The infant died after administration of the obtained substances. Specimens obtained at autopsy were analyzed and determined to contain acetaminophen, metoclopramide, and chlorpheniramine, at concentrations potentially adversely affecting the health of the child. The pharmacology of these agents as well as potential toxicity to the infant will be discussed.

Peggy Greenwald, MD, will be speaking to a case involving α-PVP and pentylone in a 1-month-old infant. These relatively new compounds to the drug scene belong to the class of substances more commonly referred to as "bath salts." Case studies involving the presence of such compounds in infants are indeed rare today. This case study will reflect on the pharmacology and toxicology of these compounds and strive to ascertain expected effects in infants.

Lucas Zarwell, MS, will discuss two cases of uncommon toxicological findings in children. The first case involves a 2-year-old that complained of "hotness." This finding was accompanied by shaking, sweating, and gasping for air. The child became non-responsive and, despite resuscitative efforts, died. Toxicological findings demonstrated a fatal concentration of chloroquine and metabolite. Chloroquine is used in children for the treatment of malaria. As little as 1g is potentially fatal in a child. The second case involves a 6-year-old who was found in seizure. She died 2 hr later and had a negative autopsy. Toxicological analyses demonstrated a fatal concentration of the atypical antidepressant bupropion. Bupropion is used in children for such conditions as ADHD. Seizures are a minor side effect of bupropion administration in children, but have been reported.

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, Toxicology, Postmortem

LW1 Martin Frobisher's Gold: Forensic Inquiry of a 16th-Century Explorer's Findings

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After attending this presentation, attendees will have an understanding of the early exploration of the search for a Northwest Passage from England to China, and of the life of an English sea captain. In addition, they will learn of the mystery of the iron blooms.

This presentation will impact the forensic science community by providing an understanding of the investigative procedures akin to the type of forensic inquiry used in the 1981 Smithsonian expedition to this area.

Martin Frobisher (1535–1594) was an English sea Captain, an Admiral in the sea battle with the Spanish Armada, and a privateer.[1] With the funding help from an English merchant consortium company, he commenced a search for the Northwest Passage to China. He sailed June 7, 1576 with three ships and 35 men. He reached Baffin Island in Northeastern Canada on August 18, 1576. He was prevented from searching further for the Northwest Passage because of heavy ice. During his time ashore, he collected a few specimens of black rocks.

Isobel, Martin Frobisher's indignant spouse of 17 years, spurned a gift of a black rock on his return from his four-month voyage and tossed it into the fireplace. When the rock was later retrieved, it sparkled "with a bright marquesset of golde," causing the start of both the largest Arctic exploration ever organized and gold fever.

A frenzy of investing groups formed, new financial paradigms were developed, and a second voyage of 145 men including miners and soldiers, arrived at the mouth of Frobisher Bay July 17, 1577. The voyagers returned on September 23, 1577 with about 200 tons of "black ore."

A third voyage started with 240 men on June 3, 1578, with 15 ships and ended October 6, during which 1,360 tons of "black ore" were collected. The "black ore" was taken to a specially built smelting plant in England and proved to be valueless. The black rock was principally Hornblende and the gold content was less than one ppb. The "marquesset of golde" was not iron pyrite, as previously thought, but was biotite.

Despite the failure to find gold, Martin Frobisher was determined to be an innocent participant and exonerated as a swindler. The guilty parties appear to be the metallurgists who performed the fire assays. The "black ore" was found to be useful for filling potholes in roads.

The interest in the Martin Frobisher voyages was reawakened by Charles Francis Hall, an American explorer who in 1861 visited Kodlunarn, or "white man's island," in Frobisher Bay. Among other items, Hall collected lumps of iron. These iron blooms were later Carbon-14 dated to 1100 A.D., centuries before Frobisher landed. For this reason, a Smithsonian expedition set out to explore Kodlunarn in August of 1981.[2] The source of these pre-Frobisher items is still a mystery.

The three voyages of Martin Frobisher failed to discover the Northwest Passage to China and India. Scholars believe this caused a loss of interest in Arctic exploration and turned British interest to colonization and exploitation of the more southern regions of the New World. Although, after the unsuccessful Arctic voyages, Martin Frobisher went on to command the largest of the Queen's ships, the Triumph, in the sea battles against the Spanish Armada.

Martin Frobisher died from a gunshot wound received while leading his marines in the attack of a Spanish garrison.

This presentation will describe the lure of gold that drove the 16th-century exploration of the Northwest area and the mystery that surrounds the artifacts left by Frobisher's adventures.

References:

1. James McDermontt, "Martin Frobisher Elizabethan Privateer", Yale, 2001
2. Fitzhugh and Olin, "Archeology of the Frobisher Voyages" Smithsonian, 1993

Frobisher, Northwest Passage, Kodlunarn

LW2 The Continuing Journey of the Mortal Remains of St. Damien: The "Leper Priest of Molokai"

Vincent J. Sava, MA, JPAC, Central Identification Lab, 310 Worchester Ave, Bldg 45, Hickam AFB, HI 96853*

After attending this presentation, attendees will appreciate St. Damien of Molokai as a historical figure and the role forensic science played in the preservation of his remains.

This presentation will impact the forensic science community by showing applications of forensic anthropology to a case involving the remains of a historical figure.

Health and disease influence the histories and cultures of any region. The legacy of leprosy in Hawaii is one of horrific and heartbreaking suffering, unimaginable hardships, as well as inspirational and heroic sacrifice, all leading to the sainthood of two individuals. One of these saints, Father Damien of Molokai, is the subject of this presentation which is also an update of a 2003 Last Word Society presentation on this topic.

A hundred years after Western contact, introduced diseases had scythed the Native Hawaiian population from 300,000 down to 50,000 people. Leprosy afflicted up to five percent of Native Hawaiians by the 1870's. To halt its spread, the Hawaiian authorities segregated patients. In 1866, several hundred patients were exiled to a "colony" on the isolated Kalaupapa peninsula on the island of Molokai. Geography makes the peninsula a natural prison. Ravaged patients were unable to climb the 2,000-foot cliffs comprising the colony's southern border or swim the rough shark-infested seas ringing its other three sides.

The early days of the colony were grim. Law and order were absent, there was inadequate shelter, little food, filthy water, scant medical care, and no hope. Half of the 800 patients arriving between 1866 and 1873 died within two years. Father Damien DeVeuster, a Belgian priest, arrived in 1873 and worked tirelessly to establish a quality of life for his patients. Father Damien died of leprosy in April 1889 at the age of 49 and was buried on Kalaupapa.

As in life, Father Damien did not rest too long in one place. In 1936, at the request of the Belgian government, Father Damien's remains were returned to his homeland. Father Damien's travels would have ended there if not for a 37-year-old French nun. Sister Simplicia Hue fell ill in February of 1895 with an acute and severely painful gastrointestinal condition—possibly ulcerative colitis, cancer, or diverticulitis. Near death, Sister Simplicia prayed for intercession by Father Damien, whose picture hung next to her deathbed. On September 11, Sister Simplicia became comatose. Early in the morning of September 12; however, she awoke pain free and ate a hearty breakfast. Sister Simplicia lived for another 32 years without any recurrence of her illness.

A sainthood cause began for Father Damien in 1938. In 1956, Belgian pathologists examined his remains as a requirement for

sainthood. In 1991, Sister Simplicia's cure was declared miraculous. Consequently, Father Damien was declared a Christian hero in 1995, resulting in another journey. Father Damien's right hand was reinterred in its original grave on Molokai.

In 1998, after praying at Father Damien's grave, Audrey Toguchi, from Hawaii, was cured of terminal cancer of the fat cells that had metastasized to her lungs. This miracle led to Father Damien's canonization as a saint on October 11, 2009 in Rome. Hundreds of Hawaiian islanders attended the ceremony. St. Damien would undertake another journey.

The Bishop of Honolulu requested from the Church in Belgium a relic of St. Damien for enshrinement in Hawaii. Rather than re-open St. Damien's crypt, officials instead selected a relic that was inexplicably left out of the crypt during the 1956 examination. The relic was a poorly preserved partial talus, side undetermined.

The relic had significantly deteriorated during its trip from Belgium to Rome and Hawaii. The Bishop requested assistance from the author regarding its conservation. The Central Identification Laboratory, Hawaii received the relic as an unofficial case on October 19, 2009. Time was donated to the conservation effort. Further deterioration was halted by treating the bone with a polymer. The relic was confirmed as a left talus.

The relic is currently enshrined in the Cathedral of Our Lady of Peace in Honolulu, thus completing, for now, the travels of the mortal remains of St. Damien.

Leprosy, Human Remains, Religious Relics

LW3 Slavery in Illinois: John Hart Crenshaw and the Old Slave House

Darlene Shelton, PhD, 3 Taliar Ridge Rd, Guilford, CT 06437*

After attending this presentation, attendees will become familiar with an elaborate interstate criminal enterprise for kidnapping, enslaving, trafficking, and breeding free African-Americans that operated in Southern Illinois and neighboring states from 1840 to 1860.

This presentation will impact the forensic science community by providing information to those who are involved with missing persons, kidnapping, human trafficking, slavery, and torture.

From 1840 to 1860, while Abraham Lincoln was establishing his reputation as a talented orator, attorney, and politician in Southern Illinois, another individual, John Hart Crenshaw was creating an interstate crime organization fueled by greed, cruelty, corrupt politics, and callous disregard for the law. Based in the town of Equality, Illinois, the criminal operation preyed on freedmen and their families drawn to Southern Illinois by fertile farmland and large communities founded by former slaves—Locust Grove in Williamson County and Miller's Grove in Pope County. Crenshaw's Gallatin County adjoined these settlements and his crime ring was dedicated to kidnapping, enslaving, and breeding his African American neighbors, including children as young as seven-years-old.

John Hart Crenshaw employed an elaborate criminal network of family and friends, using hideouts and holding stations in Western Kentucky, Southern Illinois, and an area of Missouri that stretched from St. Louis to the southern tip of the state's boot heel. He successfully laundered his criminal profits through legitimate businesses, while his occasional brushes with the law were inconsequential. Crenshaw and his wife, the former Sina Elizabeth Taylor, enjoyed a prominent place in local society. Crenshaw's wealth and land grew exponentially as he exploited the slave labor of his kidnapped victims to mine salt from the natural mineral-rich springs of present day Saline County.

As Crenshaw's interests grew, he sought to expand his political influence. He was one of the few Illinois Democrats to support a system of internal improvements, and probably conferred with one of the system's staunchest proponents, the Whig minority leader, Illinois Representative Abraham Lincoln. Local tradition says that Crenshaw held a dance in Lincoln's honor and hosted him overnight in his home, the Hickory Hill Plantation House. Built in 1838, the Hickory Hill Plantation House, known today as "The Old Slave House," still stands and is the only remaining structure of its type. The house was specifically designed to hold kidnapped African-American men, women, and children. The captives were held on the third floor, which includes a series of small wooden cells, a whipping post, and markings on the floors and walls from chains and leg irons which restrained the victims.

Crenshaw's kidnapping and slavery operation was one of the longest operating slavery rings in U.S. history. His cruelty and sexual depravity became legendary and still persist in the oral tradition of Southern Illinois.

Kidnapping, Slavery, Racketeering

LW4 Finding Amelia Earhart With Forensic Science

Richard Gillespie, BA, TIGHAR, 2812 Fawkes Dr, Wilmington, DE 19808*

The goal of this presentation is to review what The International Group for Historic Aircraft Recovery (TIGHAR) has learned in applying forensic techniques to evidence uncovered during our investigation of the Earhart disappearance. Contrary to the general public's misconception that forensic science is only of use in confirming or disproving "smoking gun" evidence, TIGHAR uses forensic analysis whenever possible to quantify aspects of the investigation that would otherwise be left to subjective judgment. In that sense, forensic findings serve as stepping stones in a sea of speculation; but, because they often direct the course of the investigation and the budgeting of scarce resources, it is important to have crucial findings verified and lab results replicated by independent, disinterested sources. It has also been learned that in some rapidly-evolving forensic disciplines such as DNA research, there are misconceptions – even among professionals – about what is and is not possible. Unrealistic optimism can burden field work with time-consuming and expensive procedures while fostering high expectations that result in unnecessary disappointment.

This presentation will impact the forensic science community by providing an example of how a nonprofit institute that is not directly affiliated with government, academia, or law enforcement is using forensic analysis to solve an iconic historical mystery.

In its ongoing investigation of the 1937 disappearance of famed female aviator Amelia Earhart, The International Group for Historic Aircraft Recovery (TIGHAR), a nonprofit aviation history institute, has drawn upon a variety of forensic disciplines in assessing evidence gathered during twenty-four years of research and ten archaeological expeditions to the uninhabited South Pacific atoll where Earhart and her navigator appear to have met their fate.

Most of TIGHAR's forensic work is done on a volunteer basis by scientists who are members of the organization. When outside expertise is needed, TIGHAR often receives assistance from government agencies and, when necessary, contracts with specialists and laboratories. Examples include:

- Forensic anthropologist and TIGHAR member, Karen Ramey Burns PhD, University of Georgia and forensic anthropologist Cecil Lewis PhD, Molecular Anthropology Laboratories, University of Oklahoma have performed forensic examination of bones and bone fragments suspected of being Earhart's. Some specimens have been eliminated. Others await further study.
- Small broken bottles recovered from a site on the island where a castaway's remains were discovered in 1940 have been identified by a variety of forensic techniques as having contained products of American manufacture marketed to women in the 1930s.

- Forensic analysis of historical photos has been key to TIGHAR's Earhart investigation, enabling TIGHAR field teams to locate important archaeological sites on the island and, most recently, evaluating a photo that may show debris from the Earhart aircraft on the island's fringing reef three months after the disappearance. Forensic imaging specialist and TIGHAR member Jeff Glickman determined that an anomalous object in the photo is consistent with the landing gear of Earhart's aircraft. The U.S. State Department Bureau of Intelligence & Research Photo Imaging Section replicated his findings.

The riddle of Amelia Earhart is being solved through the application of rigorous historical research, sound archaeological field work, and meticulous forensic examination.

Earhart, History, Aviation

LW5 Losing Your Hair in Missouri — The Centralia Massacre and Scalping by Missouri Guerillas in the American Civil War

Thomas P. Quinn, JD, 355 S Teller St, Ste 200, Lakewood, CO 80226*

WITHDRAWN

LW6 DNA and Forensic Anthropology in Trial of a Cold Case

J. Christopher Anderson, JD, 700 Adams St, Ste 250, Toledo, OH 43624*

After attending this presentation, attendees will understand the practical difficulties of a multi-jurisdiction cold case investigation, the forensic tools used in the investigation, and the evidentiary issues in a trial of a murder that occurred more than 40 years earlier.

The presentation will impact the forensic science community by providing information from a case study from the discovery of a 14-year-old girl's body until the trial and conviction of her murderer, some 44 years later.

On December 18, 1967, 14-year-old Eileen Adams left school on a public bus in Toledo, Ohio. She was expected to arrive at her sister's house at 3:30 p.m. A friend saw her on the bus but Eileen never made it to her sister's house. Forty-four days later, a hunter found her body in Monroe County, Michigan. Her body was dressed in clothing she wore to school the day she disappeared. She was hog-tied and a tenpenny nail protruded from her skull. The Monroe County coroner concluded that the homicide was caused by probable strangulation, without any mention of a hyoid bone. The investigation turned up no suspects and the case went cold.

Five years after Eileen disappeared, Margie Bowman reported to Toledo Police that in December 1967, she was living with Robert Bowman in Toledo and had recently given birth to their daughter, Brenda. She opened a door in the basement to investigate a noise and saw a young girl, naked, tied to the wall, "like Jesus." Robert threatened to kill both Margie and Brenda, and he made Margie go with him when he drove the girl's body into Michigan. Margie and Robert moved soon after, and Margie left Bowman while they were living in Miami, Florida.

After Margie's report, detectives located Bowman living in a burned out Miami restaurant. A Spiderman doll with a needle pushed between its eyes hung upside down on one wall, and a tenpenny nail was stuck in the back of the head of a Mattel® Ken doll. Interviews of Bowman failed to provide evidence sufficient to support charges and the case went cold again.

In September 2006, the Toledo Cold Case Unit reopened the case. A partial DNA profile was obtained from semen in Eileen's underwear, but Bowman's profile was not in CODIS. However, a reverse paternity test compared Brenda's profile to the sample obtained from Eileen's underwear. The test results indicated that Bowman could not be excluded as the source of the DNA and a warrant was issued for his arrest. In 2008, he was located in Riverside, California where he was homeless. He waived extradition and was returned to Ohio.

Before trial, Eileen's remains were exhumed. The hyoid bone was not found in the exhumation. Three forensic anthropologists concurred that Eileen had at least five blunt force injuries to her head. The injury to her forehead was the most severe, causing a linear fracture that ran the entire circumference of her skull. The anthropologists concluded that she died of blunt force trauma.

The trial was held in August 2011 but resulted in a hung jury. Bowman was convicted when he was retried and at the age of 76 was sentenced to life in prison.

DNA, Forensic Anthropology, Cold Case

LW7 A Producer's Wife, One Cliff in Malibu, and a Decapitated Head — Two Cases Utilizing Forensic Art

Sandra R. Enslow, BA, 4700 Ramona Blvd, Rm LL40, Monterey Park, CA 91754*

After attending this presentation, attendees will understand some of the various aspects of the forensic art discipline and its function in a criminal investigation.

This presentation will impact the forensic science community by raising awareness of the forensic art discipline. Two very different cases will illustrate the use of forensic art, both under difficult circumstances. The first case presented is a composite that corroborated identification of the suspect; and the second case, a postmortem drawing that assisted in the identification of the victim of decapitation. Though it is ideal for a forensic composite to elicit an outright identification, they do not need to identify a suspect directly to be successful. Many forensic composite artists work in the field, in the media, in the public, and in the courtroom for the jury.

The first case involves the victim of an attempted rape. Out for an early morning jog along the cliffs, a woman, a mother and wife of a TV producer, stopped to sit and meditate with the rising sun, when she was attacked. After a lengthy struggle with her attacker, she was able to get near the cliff. Rather than face what the attacker was offering, she made the decision to take the plunge off the cliff (100') in Malibu, California – leaving her car keys behind. She survived the fall but gave up her car to her assailant. DNA found on a tequila bottle at the crime scene would ultimately identify the suspect. The conditions under which the drawing was created were not ideal, yet the victim was willing and eager to describe the assailant, despite her injuries. The final composite had similarities to the booking photo and played a supporting role in the courtroom as jurors considered all of the evidence before them. The forensic drawing did not elicit a direct identification but supported the other disciplines in the investigational process. The victim, the local media, and ultimately the jury saw the likeness and agreed.

The second case presents a 1995 homicide in the Santa Clarita Valley region of Southern California. The investigation required a postmortem forensic drawing, not a composite, to be created. The forensic image, created from the views of the victim's decapitated head, ultimately allowed a sister to recognize and subsequently identify her missing and troubled younger brother. This is the case of Frederick Walker, who was beaten, beheaded, and dismembered by two of his associates for $635.00. The course of this very convoluted case includes U.S. Forest Service undercover agents and a fifteen-year-old girl and her mother. The Los Angeles County Sheriff's

Department forensic artist testified to her work in each defendant's trial. After both trials were over, the victim's sister met with the detective and forensic artist. Since she didn't have a picture of her brother as an adult, and felt the forensic drawing was an accurate reflection of him, she asked if the family could have it.

This presentation will examine the interesting turns in each of these cases. How forensic art supported the investigations, in the field and in the courtroom, will be highlighted.

Forensic Composite, Postmortem Drawing, Homicide

LW8 The Titanic Identification Project: Lessons Learned

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The goal of this presentation is to illustrate the multifaceted nature of historic investigations highlighting the importance and problems of mitochondrial DNA (mtDNA) testing in such cases.

This presentation will impact the forensic science community by showing how mtDNA testing holds the key to final identification of unknown human remains when there is sufficient verifiable DNA and compliant known mitochondrially related relatives.

Hypothesis: mtDNA testing holds the key to final identification of unknown human remains when there is sufficient verifiable DNA and compliant known mitochondrially related relatives.

Methods: Pedestalling excavation techniques, dental aging, and amplification and sequencing of mtDNA in both the coding and non-coding regions of the mitochondrial genome.

All forensic cases, including those of historic interest, begin at the scene. In May of 2001, permission was obtained to exhume and identify three bodies from the Titanic disaster buried in the Fairview cemetery in Halifax, Nova Scotia, using mtDNA. As part of this research living relatives of the targeted individuals were identified and permission to secure their mtDNA was also obtained. The genealogical sleuthing was carried out long before the excavation. After failing to obtain any biological material from two adult skeletons where a backhoe was used directly over the bodies, the potential of DNA recovery from a child burial (#4) seemed remote. The decision to pedestal the child burial was made despite time restrictions. The change in excavation strategy was rewarded by the recovery of a long bone fragment and several tooth buds. The fact that the organic remains were submerged in water did not auger well for the preservation of mtDNA despite the fact that the remains had been interred for less than a century (April of 1912). Historically, "burial 4" had always been assumed to be Gösta Pålsson a 28-months-old Swedish boy. Immediately, the examination of the tooth buds for damage clearly indicated a much younger individual, approximately 1 year $\pm$3 months of age using Caucasian dental standards, and verified in blind by three experts. This information was withheld (blind design) from the DNA part of the investigation. Surprisingly, DNA was amplified both from the bone and dentin with the HV1 and HV2 regions of the mitochondrial genome being targeted. Of six children who could potentially be identified as "burial 4", two individuals, Eino Panula a Finish child aged 13 month and a British child Sidney Goodwin aged 19 months, were clearly the closest fit with the dental and non-biological (shoe) evidence. Both had the same and common HV1 and HV2 Caucasian haplotypes. In a PBS documentary, the Titanic Identification Project's final puzzle was aired with the Panula family placing a reef of flowers around the grave of the now known child. However, several aliquots from the dentin of "burial 4" were saved and continued DNA testing for SNPs from the non-coding region by the Armed Forces DNA Identification Laboratory resulted in Goodwin being identified as the unknown child via a rare SNP (T9923C?). Many lessons were learned through this collaborative research program:

- Despite time restrictions using a proper recovery method was fundamental for the whole project – no sample, no testing!
- Assume nothing – water destroys DNA but in this case even with fragmentary from a sub-adult verifiable mtDNA was obtained by several labs.
- Dental standards have to be population specific, and with single samples, the standard deviations are just a guideline. The Goodwin child was outside two standard deviations.
- All research, where possible, should be done in blind, an obvious but often overlooked aspect of forensic research.
- With common mtDNA HV1 and HV2 haplotypes the use of variation in the non-coding regions is fundamental and should possibly be mandated for all forensic testing.

Conclusions: This project shows the problems associated with making assumptions based on previous research (e.g., can't get DNA from waterlogged samples) and the importance of continued DNA testing especially when dealing with common mtDNA haplotypes.

mtDNA, Excavation, Aging

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Siemens Healthcare Diagnostic, Inc. (Discussion of Commercial Products or Services)
NMS Labs (Employee)
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Federal Government (Employee)
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3M Incorporated, ESPE AG, Renishaw, University College London (Discussion of Commercial Products or Services)
Zainah N. Al Hatali, BSc - D75
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Osamah A. Al Hmoudi, BSc - D75
SIMUL8 Corporation (Discussion of Commercial Products or Services)
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SIMUL8 Corporation (Discussion of Commercial Products or Services)
Khudooma S. Al Na'imi, MSc - D75
SIMUL8 Corporation (Discussion of Commercial Products or Services)
Mariam A. Al Qahtany, MSc - D75
SIMUL8 Corporation (Discussion of Commercial Products or Services)
Mahmoud B. Al Sharairi, BSc - D75
SIMUL8 Corporation (Discussion of Commercial Products or Services)
Joseph Alape, MD - D13
Applied Biosystems, Inc., PROMEGA (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device)
A. Midori Albert, PhD - H100
Discloses no financial relationships with commercial entities.
Ellas Aldrich, BS - A150
University of Alabama (Other Financial/Material Support)
Adam K. Aleksander, PhD - C28, C33
Discloses no financial relationships with commercial entities.
Cierra I. Alexander - J8
Discloses no financial relationships with commercial entities.
Stuart E. Alexander, DMD - F24
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Alberto Amadasi, MD - H33
Discloses no financial relationships with commercial entities.
Michael T. Ambrosino, JD - E6, S1
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
James N. Anasti, BS - K6
Discloses no financial relationships with commercial entities.
Bruce E. Anderson, PhD - H80
Discloses no financial relationships with commercial entities.
Dan T. Anderson, MS - S2
Discloses no financial relationships with commercial entities.
Gail S. Anderson, PhD - H118
Canadian Police Research Centre, Victoria Experimental Network UnderSea (Grant Support)
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Discloses no financial relationships with commercial entities.
John A. Anderson, PhD - H104
Discloses no financial relationships with commercial entities.
Robert N. Anderson, PhD - LW1
Discloses no financial relationships with commercial entities.
Scott A. Anderson, BS - C11
Livermore Software Technology Corporation (Discussion of Commercial Products or Services)
Forensic Engineering, Inc. (Employee)
Sebastien Anizan, PhD - K52
ThermoFisher (Discussion of Commercial Products or Services), National Institutes of Health, Intramural Research Program, NIDA (Employee)
Diana Aparicio, DDS - F12
Servicio Médico Legal (Employee)
Isabella Aquila, MD - C1, I5, I32, G21, G22, G23, G40, G41, G42, G108, G109, G110, K23
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Drager (Discussion of Commercial Products or Services)
The Center for Forensic Science Research and Education (Other Financial/Material Support)
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Minnesota Public Defender's Office (Employee)
Richard Ayers, MS - B31
NIST (Employee)

B

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Discloses no financial relationships with commercial entities.
Sarah E. Bahlmann, BS - G2
Discloses no financial relationships with commercial entities.
James A. Bailey, PhD - D19
JEOL USA, Gilbert Smith (Discussion of Commercial Products or Services)
JEOL USA (Discussion of Unlabeled/Investigational Use of Product/Device)
Andrew M. Baker, MD - W11
Discloses no financial relationships with commercial entities.
Charlotte A. Baker, PhD - K66
Dolgen, Corp., DuPont, Falcon Safety Products, Inc., PowerMaster (Discussion of Commercial Products or Services)
Lori E. Baker, PhD - H130
Lucas Grant, Forensic Sciences Foundation, Inc. (Grant Support)
Shankar M. Bakkannavar, MD - K63
Discloses no financial relationships with commercial entities.
Alicia L. Bales, MD - I36, I37
Discloses no financial relationships with commercial entities.
Manal S. Bamousa, MD - I7
Discloses no financial relationships with commercial entities.
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Life Technologies (Discussion of Commercial Products or Services)
National Institute of Justice (Grant Support)
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University of New Haven (Other Financial/Material Support)
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Discloses no financial relationships with commercial entities.
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National Institute of Justice (Grant Support)
Bruker Corporation, Craic (Discussion of Unlabeled/Investigational Use of Product/Device)
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Discloses no financial relationships with commercial entities.
Michael Baumann, PhD - W19
Discloses no financial relationships with commercial entities.
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Envisage Systems, Foster+Freeman, Keyence, Kubtec, Leica Microsystems (Discussion of Commercial Products or Services) - S2
Foster+Freeman (Discussion of Commercial Products or Services) - J21
U.S. Immigration and Customs Enforcement (Employee) - J21
Melanie M. Beasley, MA - H131
Discloses no financial relationships with commercial entities.

Rachel C. Beck, BS - K25
DPX Labs, JEOL USA (Discussion of Commercial Products or Services)
Stefan Becker, PhD - A113
Flachglas, Herzogenrath, Libbey Owens Ford, Vegla (Discussion of Commercial Products or Services)
Bundeskriminalamt, German Federal Criminal Police Office (Employee)
Matthew Begert, BS - C10
The Aerospace Corporation (Employee)
George S. Behonick, PhD
Diggnew.com, Ivory Wave, Myremedys.com, Pure-Bath.com, Vanilla Sky Wholesale (Discussion of Commercial Products or Services) - K3
Dell Publishing, Exit International U.S. (Discussion of Commercial Products or Services) - G148
Discloses no financial relationships with commercial entities. – W6
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Office of the Minnesota State Public Defender (Employee)
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Canadian Police Research Centre, Victoria Experimental Network UnderSea (Grant Support)
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West Virginia University (Employee) - A110
Raffaello M. Bellino, MD - G145
Genentech/Roche (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
David M. Benjamin, PhD - K44
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Discloses no financial relationships with commercial entities.
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DNA Polymerase Technologies (Discussion of Commercial Products or Services, Other Financial/Material Support, and Discussion of Unlabeled/Investigational Use of Product/Device)
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Comedy Central, Comic Strip Live, Gotham Comedy Club, St. Martin's Press (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Jurrien Bijhold, PhD - W17
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Roche Diagnostics (Discussion of Commercial Products or Services)
National Institute of Justice (Grant Support)
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Sandra C. Bishop-Freeman, PhD - K9
Pfizer (Discussion of Commercial Products or Services)
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Abacus Diagnostics, BlueStar Forensic, Tink's (Discussion of Commercial Products or Services)
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George Gershwin (Discussion of Commercial Products or Services)
Alessandro Bonsignore, MD - G129
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities - H89
Carcano, Mauser, Moschetto, Steyr-Mannlicher, Vetterli-Vitali (Discussion of Commercial Products or Services) - D18
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Keyence Corporation, Inc. (Discussion of Commercial Products or Services)
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DHS (Grant Support)
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Restek Corporation (Discussion of Commercial Products or Services)
Pennsylvania State University (Grant Support)
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Foster+Freeman (Discussion of Commercial Products or Services)
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Federal Bureau of Investigation (Employee)
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Leica Microsystems, TransCon Imaging Solutions (Discussion of Commercial Products or Services)
TransCon CSI – Crash Scene Investigations (Employee)
Leica Microsystems (Discussion of Unlabeled/Investigational Use of Product/Device)
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Simon & Schuster, Inc. (Discussion of Commercial Products or Services)
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Applied Biosystems, Inc., Illumina, Qiagen (Discussion of Commercial Products or Services)
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Life Technologies, National Institute of Standards and Technology, Promega Corporation (Discussion of Commercial Products or Services)
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Life Technologies, Qiagen (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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JPAC/CIL (Employee) - W10
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JPAC/CIL (Employee)

C

Luciana Caenazzo, PhD - A134, D1
Discloses no financial relationships with commercial entities.
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Applied Biosystems, Inc., Promega Corporation, Qiagen (Discussion of Commercial Products or Services)
Michael Caligiuri, PhD - W4
Neuroscrippsoftware.com, Wacom (Discussion of Commercial Products or Services)
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Amicon, Promega Corporation, Qiagen (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Craigslist (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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University of Strathclyde and Cascade Technologies (Grant Support)
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Discloses no financial relationships with commercial entities.
John J. Carney, JD - E28
Cellebrite/UFED, Katana Forensics/Lantern, Oxygen Forensics, Timeline Tools (Discussion of Commercial Products or Services)
Carney Consulting, LLC (Shareholder)
Andrew E. Carson, MFS - D40
Discloses no financial relationships with commercial entities.
David O. Carter, PhD - G138
Discloses no financial relationships with commercial entities.
Daylien Casas, BS - I34
Neogen Corporation (Discussion of Commercial Products or Services)
Brent A. Casper, BS - A139
Shimadzu, Sigma-Aldrich (Discussion of Commercial Products or Services)
Shannon Cassatt, MS - K15
Ortho-McNeil-Janssen Pharmaceuticals, Inc. (Discussion of Commercial Products or Services)
Joseph I. Cassilly, JD - E9
Discloses no financial relationships with commercial entities.
Glorianna Caves, BS - G52
Copan Italia (Discussion of Commercial Products or Services)
Joseph S. Cecil, PhD - S1
Discloses no financial relationships with commercial entities.
Salih Cengiz, PhD - J1
Discloses no financial relationships with commercial entities.
Michelle Cerreta, BS - A15
Discloses no financial relationships with commercial entities.
Robin P. Cessna, MFS - J29
Foster+Freeman (Discussion of Commercial Products or Services),
HSI Forensic Laboratory (Employee)
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Discloses no financial relationships with commercial entities.
William L. Chapin, BA - W3
Discloses no financial relationships with commercial entities.
Rosa E. Chapman, MS - D21
Abacus Diagnostics, Independent Forensics, SERI (Discussion of Unlabeled/Investigational Use of Product/Device)
Carole E. Chaski, PhD - I23
ALIAS Technology, LLC (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device)
Luis Chavez, BS - C31
CBC, Federal, Italo Gra, Mauser, Remington, Savage, Sig Sauer, Smith and Wesson, TEC (Discussion of Commercial Products or Services)
Policia de Investigaciones de Chile (Employee)
Chun-Yu Chen, MS - A200
Affiniton, LLC (Discussion of Commercial Products or Services)
Michael Chen, PhD - K54, W12
Discloses no financial relationships with commercial entities.
Xinshan Chen, PhD - G113
Discloses no financial relationships with commercial entities.
Christine Chevallier - G64
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Shaylin P. Chock, MD - I4
Discloses no financial relationships with commercial entities.
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Supelco Analytical, Waters (Discussion of Commercial Products or Services)
Shivani Chopra, MD - I27
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Angi M. Christensen, PhD - H57
Rofin Forensic (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Renaud Clement, MD - G78, G86
Discloses no financial relationships with commercial entities.
Michael D. Coble, PhD - A128
Life Technologies, Promega Corporation (Discussion of Commercial Products or Services)
National Institute of Justice (Other Financial/Material Support)
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Discloses no financial relationships with commercial entities- F6
Adobe Systems, Inc. (Discussion of Commercial Products or Services) - F9

Thomas Colard, DDS, PhD
Brenneke (Discussion of Commercial Products or Services) - H97
Agence National de la Recherche (Grant Support) - H97
Discloses no financial relationships with commercial entities - F19
Valerie L. Colley, BS - A24
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Aglient Technologies, Applied Biosystems, Inc., Princeton Separations, Qiagen (Discussion of Commercial Products or Services)
Kim A. Collins, MD - W8, W22
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Discloses no financial relationships with commercial entities.
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PharmCheck, Sarstedt (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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JPAC-CIL, Oak Ridge Institute for Science and Education (ORISE) (Grant Support)
Aime H. Conigliaro, MA - F15
Discloses no financial relationships with commercial entities.
Gerald J. Conlogue, MHS - D34
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Abacus Diagnostics, Seratec (Discussion of Commercial Products or Services)
NMS Labs (Employee)
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Discloses no financial relationships with commercial entities.
Graziamaria Corbi, PhD - I35
Discloses no financial relationships with commercial entities.
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Apple, Inc., Access Datagroup, LLC, Cellebrite Mobile Synchronization, Ltd., Zynga, Facebook®, Inc., HTC (Discussion of Commercial Products or Services)
Target Corporation (Employee)
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Coulter, Waters (Discussion of Commercial Products or Services) - G50, H40, K5
Angelique Corthals, PhD - S2
Discloses no financial relationships with commercial entities.
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NMS Labs (Employee)
Gerard M. Courtin, PhD - G157
Discloses no financial relationships with commercial entities.
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Elizabeth Cowper, BS - C15
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Discloses no financial relationships with commercial entities.
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Cooperative Agreement with the National Institute of Justice (Grant Support)
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Millipore, Sartorius Stedim (Discussion of Commercial Products or Services)
NMS Labs (Employee)
Christian Crowder, PhD - H61, W11
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Discloses no financial relationships with commercial entities.
Sharon Rose Crowley, MN - G116
Discloses no financial relationships with commercial entities.
Eugenia Cunha, PhD - H2
Discloses no financial relationships with commercial entities.
Barry Cushman, BS - D55
Axcition Systems, Inc. (Discussion of Commercial Products or Services)

D

Gretchen R. Dabbs, PhD - H113
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Discloses no financial relationships with commercial entities.
Franklin E. Damann, PhD - H109
National Institute of Justice (Grant Support)
Caitlin N. Dardenne, MS - H128
Discloses no financial relationships with commercial entities.
Thomas J. David, DDS - E48, S2
Discloses no financial relationships with commercial entities.
Elizabeth H. Davies, BS - E35
National Institute of Justice (Grant Support)
Joshua Davis - A27
Forensic Technology, Inc., Leica Microsystems, Microsoft Corporation, Mossberg & Sons, Inc., Norsys Software Corp., Remington Arms Company, LLC, Walsh Automation (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Life Technologies, Promega Corporation (Discussion of Commercial Products or Services)
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Applied Biosystems (Discussion of Commercial Products or Services)
IUPUI (Other Financial/Material Support)
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Discloses no financial relationships with commercial entities.
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Oak Ridge Institute for Science Education (Grant Support)
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Discloses no financial relationships with commercial entities.
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George Mason University (Other Financial/Material Support)
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National Institute of Justice (Grant Support)
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National Institute of Justice (Grant Support)
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Discloses no financial relationships with commercial entities.
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Applied Biosystems (Discussion of Commercial Products or Services)
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E

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Restek Corporation, Shimadzu Scientific, Inc. (Discussion of Commercial Products or Services)
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Thermo Electron Corporation (Discussion of Commercial Products or Services)
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Smiths Detection (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
Haley A. Elliott, MFS - J4
FiberStone, Gartner, Inc., Natural Source Printing, Oxford, Terraskin, ViaStone Corporation (Discussion of Commercial Products or Services)
USPIS (Employee)
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CBC, Federal, Italo Gra, Mauser, Remington, Savage, Sig Sauer, Smith and Wesson, TEC (Discussion of Commercial Products or Services)
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Z Corporation (Discussion of Commercial Products or Services, Other Financial/Material Support, and Discussion of Unlabeled/Investigational Use of Product/Device)
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Almay, Inc., Avalon Natural Products, Avon Products, Inc., The Bonne Belle Company, Boots USA, Burt's Bees, CBI Disturbing Corp., Clarins, Clinique Laboratories, LLC, Coty U.S., LLC, Elizabeth Arden, Inc., Estée Lauder, Inc., Garden Botanika, H2O Plus, L.P., Jean Philippe Fragrances, LLC, LANCÔME, Lee Pharmaceuticals, L'Oreal USA, Inc., Make-Up Art Cosmetics, Markwins Beauty Products Inc., Mary Kay, Maybelline, LLC, NARS Cosmetics, Neutrogena Corporation, NYX Cosmetics, Palladio Beauty, Procter and Gamble, Revlon, Inc., The Topps Company, ULTA Salon, Cosmetics & Fragrance, Inc., Victoria's Secret (Discussion of Commercial Products or Services)
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Zeno J. Geradts, PhD
Facebook®, Inc., Google, Yahoo! Inc. (Discussion of Commercial Products or Services - B3
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PRNU Compare (Discussion of Unlabeled/Investigational Use of Product/Device) - B3
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Taylor N. Grieve, BS - C14
Brown Bear, CCI, Cor-Bon, Federal-American Eagle, Hi-Point, Independence, PMC, Remington, Sellier & Bellot, Sig Sauer, Silver Bear, Taurus (Discussion of Commercial Products or Services)
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Florida Spice, Florida Spice Melon, K2 420 Summit, K2 Blonde, K2 Cloud 9, K2 Melon, Kush, Matrix Platnium, Mr. Kwik E, Purple Flake (Discussion of Commercial Products or Services)
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Wacom Co., Ltd. (Discussion of Commercial Products or Services)
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National Institute of Justice (Grant Support)

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Applied Biosystem, Fitzco, Inc., Promega Corporation (Discussion of Commercial Products or Services)
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Glock, Ges.m.b.h. (Discussion of Commercial Products or Services)
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CZ Corporation, Dan Wesson Firearms (Discussion of Commercial Products or Services)
Forensic Sciences Foundation, Inc. (Grant Support)
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Phusion Projects, LLC (Discussion of Commercial Products or Services)
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Facebook®, Inc., Gourmet, Food & Wine, GQ, Harper Collins Publishers, Martha Stewart Living, New York Magazine, New York Times, Tumblr, Twitter, WordPress (Discussion of Commercial Products or Services) - BS5
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AB SCIEX, Stat-Sure, Thermo Fisher Inc. (Discussion of Commercial Products or Services) - K50
NIDA (Employee) - W19
National Institutes of Health, Intramural Research Program, NIDA (Employee and Discussion of Unlabeled/Investigational Use of Product/Device) - K50
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Gift of Life Michigan, Ora, Inc. (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device) - G111
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Pfizer (Discussion of Unlabeled/Investigational Use of Product/Device)
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Smiths Detection (Discussion of Commercial Products or Services)
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Clarity Medical Systems, Inc. (Discussion of Commercial Products or Services) - G82
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National Institute of Justice (Employee) - BS1
Sabreena Larson, MS - A192
Discloses no financial relationships with commercial entities.
Gregory E. Laskowski, MPA - A193
Discloses no financial relationships with commercial entities.
Jared Latiolais, MSc - A40
Roche (Discussion of Commercial Products or Services)
TSWG (Grant Support)
Nancy Laurin, PhD - A85
BioRad, Life Technologies, Promega Corporation, Qiagen, Takara Bio, Tecan Group, Ltd. (Discussion of Commercial Products or Services)
Eric S. Lavins, BS - K35
Agilent Technologies, AIT Laboratories, Restek Corporation, Waters Corporation
(Discussion of Commercial Products or Services)
Eric Law - A27
Forensic Technology, Inc., Leica Microsystems, Microsoft Corporation, Mossberg & Sons, Inc., Norsys Software Corp., Remington Arms Company, LLC, Walsh Automation (Discussion of Commercial Products or Services)
Apisri Leamniramit, BS - K74
DPX Labs (Discussion of Commercial Products or Services)
Marc A. LeBeau, PhD - E56, W14
Federal Bureau of Investigation Laboratory (Employee)
Igor K. Lednev, PhD
Discloses no financial relationships with commercial entities - A108
National Institute of Justice (Grant Support) - A34
Dayong Lee, MS - K49
Immunalysis, Inc. (Discussion of Commercial Products or Services)
NIDA, NIH (Employee)
GW Pharmaceuticals (Discussion of Unlabeled/Investigational Use of Product/Device)
Eugene Lee, MD - I28
Discloses no financial relationships with commercial entities.
JoJo Lee, MA - I41
Discloses no financial relationships with commercial entities.
Josh D. Lee, JD - E41
Discloses no financial relationships with commercial entities.
Sangki Lee, PhD - K21
Discloses no financial relationships with commercial entities.
Suji Lee, BFA - J11
Los Angeles Sheriff's Department (Grant Support)
U-Young Lee, MD - H3
National Research Foundation of Korea (Grant Support)
Carrie B. LeGarde, MA - H46
Discloses no financial relationships with commercial entities.
Samuel J. Leistedt, MD, PhD - I19
Discloses no financial relationships with commercial entities.
Nikolas P. Lemos, PhD - K38, K42, K62, K69
City and County of San Francisco, International Federation of Professional and Technical Engineers - Local 21 (Employee)
Stephen Lenfest, MD - G16, G69
Discloses no financial relationships with commercial entities.
Iana Lesnikova, MD, PhD - G12
Roche (Discussion of Commercial Products or Services)
Danielle C. Levron, BS - G153
BoR (Grant Support)
Ryan Lewis, BS - B26
Federal Bureau of Investigation (Employee)
Zheng-Hua Li, PhD - D3
National Institute of Justice (Grant Support)
Gary A. Licht, MS - J18
Discloses no financial relationships with commercial entities.
Laura J. Liddicoat, BS - W6
Discloses no financial relationships with commercial entities.
Leandi Liebenberg, BSc - H53
Discloses no financial relationships with commercial entities.
Sze-Wah Lin, MPhil - G104
Discloses no financial relationships with commercial entities.
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Adobe Systems, Inc. (Discussion of Commercial Products or Services)
Sandra R. Lines, BA - J19
Staples (Discussion of Commercial Products or Services)
Jason G. Linville, PhD - A149
Discloses no financial relationships with commercial entities.
Laura L. Liptai, PhD - S1
Discloses no financial relationships with commercial entities.
Liang Liu, PhD - G112
Huazhong University of Science and Technology (Grant Support)
Ning Liu, MA - J31
Discloses no financial relationships with commercial entities.
Simon Liu, HBSc - A133
Hanna Instruments (Discussion of Commercial Products or Services)
Peel Regional Police, University of Toronto (Employee)
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Apple, Inc. (Discussion of Commercial Products or Services)
Department of Homeland Security, National Institute of Standards and Technology (Employee)
Cristian F. Lizama, BS - C31
CBC, Federal, Italo Gra, Mauser, Remington, Savage, Sig Sauer, Smith and Wesson, TEC (Discussion of Commercial Products or Services)
Policia de Investigaciones de Chile (Employee)
Randall Lockwood, PhD - D56
ASPCA (Employee)
Barry K. Logan, PhD - E18, K34, W19
NMS Labs (Employee)
Barbara K. Lograsso, PhD - C13
National Institute of Justice (Grant Support)
Kirk Lohmueller, PhD - W13
California Association of Criminalists, Miller Research Institute, UC Berkeley (Grant Support)
Nicolene Lottering, BS - H74
INUS Technology Inc., Visage Imaging GmbH (Discussion of Commercial Products or Services)
Jennifer C. Love, PhD - H34, W11
Harris County Institute of Forensic Sciences (Employee)
Jessica M. Lowney, BS - A68
Qiagen (Discussion of Commercial Products or Services)
National Institute of Justice (Grant Support)
Kayla Lowrie, MS - K19
National Safety Council (Grant Support)
Douglas M. Lucas, DSc - A180
Merriam-Webster, Inc. (Discussion of Commercial Products or Services)
Melania Lugli, PhD - I10, W9
Discloses no financial relationships with commercial entities.

Christine M. Lundblad, BS - A7
Federal Bureau of Investigation, Oak Ridge Institute for Science and Education (Other Financial/Material Support)
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Restek Corporation (Discussion of Commercial Products or Services)
The Pennsylvania State University (Grant Support)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.

M

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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities – D83.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Mathworks, Inc. (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Holland Maness, DMD - F2
3DMD Imaging Systems, American Journal of Orthodontics & Dentofacial Orthopedics, American Association of Orthodontics, The Clinics, Elsevier, American College of Prosthodontists, International Journal of Oral & Maxillofacial Surgeons (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Emiliano Maresi - G36, G120
Discloses no financial relationships with commercial entities.
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University of Tennessee, Knoxville (Employee)
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Air Force Research Laboratory, MIT Lincoln Laboratory (Discussion of Commercial Products or Services)
Federal Bureau of Investigation (Employee)
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Meigs County General Health District, Meigs County Coroner's Office (Employee)
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Companhia Brasileira de Cartuchos, FEI Company, Ted Pella, Inc. (Discussion of Commercial Products or Services)
FINEP (Grant Support)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Hamilton Robotics, Tecan Group Ltd. (Discussion of Commercial Products or Services)
AFDIL (Employee)
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Leica Microsystems, TransCon Imaging Solutions (Discussion of Commercial Products or Services)
University of Maryland (Employee)
Leica Microsystems (Discussion of Unlabeled/Investigational Use of Product/Device)
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Discloses no financial relationships with commercial entities.
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Fitzco Inc. (Discussion of Commercial Products or Services andDiscussion of Unlabeled/Investigational Use of Product/Device)
University of Tennessee (Grant Support)
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BenzoFury.com (Discussion of Commercial Products or Services)
National Institute of Justice (Grant Support)
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Roche, Roche Nimblegen (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Illumina, Inc. (Discussion of Commercial Products or Services)
Department of Defense, Commonwealth of Virginia (Other Financial/Material Support)
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Ford Motor Company, Mossberg & Sons, Inc. (Discussion of Commercial Products or Services)
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Eigenvector Research Inc., MathWorks, Renishaw (Discussion of Commercial Products or Services), NIJ grant (Grant Support)
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Discloses no financial relationships with commercial entities.
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McShane Law Firm (Shareholder) - W14
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R Development Core Team, University of Tennessee (Discussion of Commercial Products or Services)
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Illumina, Inc. (Discussion of Commercial Products or Services)
National Institutes of Justice (Grant Support)

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Meharry Medical College (Other Financial/Material Support)
Sharyn E. Miller, BS - K79
Pennsylvania State University (Employee)
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MVA Scientific Consultants (Employee)
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California State University (Employee)
Kevin P.C. Minbiole, PhD - K72
Villanova University (Employee)
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Izhmash (Discussion of Commercial Products or Services) - G26
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Discloses no financial relationships with commercial entities.
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San Francisco Office of the Chief Medical Examiner (Employee)
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Nerroscript Software, Wacom (Discussion of Commercial Products or Services)
J.E. Molto, PhD - LW8
PBS (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Permegear, Smiths Detection, Specialty Silicone Products (Discussion of Commercial Products or Services)
Department of Defense (Grant Support)
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Discloses no financial relationships with commercial entities.
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National Institute of Justice (Grant Support)
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Discloses no financial relationships with commercial entities.
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New York City Office of Chief Medical Examiner (Employee)
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Life Technologies (Discussion of Commercial Products or Services and Employee)
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George Washington University (Other Financial/Material Support)
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Discloses no financial relationships with commercial entities.
Martha W. Murphy, MA - L1
National Archives and Records Administration (Employee)
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Agilent Technologies, IonSense, Inc. (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.

N

Gary H. Naisbitt, PhD - A8, A136
Discloses no financial relationships with commercial entities.
Hiroaki Nakahara, PhD - A48
Fluidigm, Third Wave Japan (Discussion of Commercial Products or Services)
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Federal Bureau of Investigation (Employee)
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Discloses no financial relationships with commercial entities.
Kara L. Nance, PhD - B32
Discloses no financial relationships with commercial entities.
Stephen P. Nawrocki, PhD - H106
Discloses no financial relationships with commercial entities.
Margherita Neri, MD, PhD - G130
Discloses no financial relationships with commercial entities.
Peter Neufeld, JD - E6
Discloses no financial relationships with commercial entities.
Cedric Neumann, PhD - A217
National Institute of Justice (Grant Support)
Nicholas K. Ng, BS - B14
Apple, Facebook®, Inc., HTC, LG, Motorola, Nokia, RIM, Samsung, Specific Media, Tumblr, Twitter (Discussion of Commercial Products or Services)
Justin T. Nguyen, BS - D65
Apple, Inc., HTC, LG, Motorola, RIM, Samsung (Discussion of Commercial Products or Services)
Michael J. Nichols, JD - W5
Discloses no financial relationships with commercial entities.
Nicole M. Nicklow, MSFS - W23
Discloses no financial relationships with commercial entities.
Emily R. Niespodziewanski, BA - H126
Adobe Systems, Inc., Canon, General Electric, R Development Core Team, National Agriculture Research Organization, NextEngine, Sourceforge.net (Discussion of Commercial Products or Services)
John Nixon, MBA - C23, C24, C29
Discloses no financial relationships with commercial entities.
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Pressure BioSciences Inc., Promega Corporation (Discussion of Commercial Products or Services)
National Institute of Justice (Grant Support)
Carla M. Noziglia, MS - S1, S2
Discloses no financial relationships with commercial entities.
Jacqueline Nunez, MD - G46
Discloses no financial relationships with commercial entities.
Emilio Nuzzolese, PhD - F5, F23
Discloses no financial relationships with commercial entities.

O

Elizabeth C. Okrutny, MA - H52
Discloses no financial relationships with commercial entities.
Rodrigo B. Oliveira, PhD - A22
Finep and Inmetro (Employee)
Laura C. Oliver, MS - A44
Lonza Group, Ltd., Qiagen (Discussion of Commercial Products or Services)
Bureau of Alcohol, Tobacco, Firearms, and Explosives, Virginia Commonwealth University (Other Financial/Material Support)

William R. Oliver, MD
Discloses no financial relationships with commercial entities - W17
East Carolina University (Employee) - G59
Larry A. Olson, MFS - J22
3M Incorporated, Adobe Systems, Inc., Foster+Freeman, Microsoft Corporation (Discussion of Commercial Products or Services)
Bobbi Jo O'Neal, BSN - D50
Discloses no financial relationships with commercial entities.
Anthony J. Onorato, MS - W23
Discloses no financial relationships with commercial entities.
Janice J. Ophoven, MD - W22
Discloses no financial relationships with commercial entities.
Kei A. Osawa, BS - A16
Abacus Diagnostics, Inc., The Timberland Company (Discussion of Commercial Products or Services)
Antonio M.M. Osculati, MD - G19, G90
Discloses no financial relationships with commercial entities
Antonio M.M. Osculati, MD - G91
Emitech, Inc., FEI-Philips (Discussion of Commercial Products or Services)
Charity G. Owings, BS - G159
Discloses no financial relationships with commercial entities.

P

Christopher S. Palenik, PhD - A115
National Institute of Justice (Grant Support)
Andri Palla, BA - D26
Committee on Missing Persons in Cyprus (Other Financial/Material Support)
Cristian Palmiere, MD - G135
Discloses no financial relationships with commercial entities.
Laura Panata, MD - D67, D68
Discloses no financial relationships with commercial entities.
Hope E. Parker, BS - A67
Applied Biosystems, Inc., Qiagen (Discussion of Commercial Products or Services)
Virginia Commonwealth University (Employee)
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C.A. Pound Human Identification Laboratory (Employee)
William Parra, MSc - D13
Discloses no financial relationships with commercial entities.
M. Neal Parsons, MS - D28
Abacus Diagnostics, BLUESTAR, Foster+Freeman, Safariland, LLC, Sirche, SPEX Forensics, Tri-Tech Forensics (Discussion of Commercial Products or Services)
The Bode Technology Group (Other Financial/Material Support and Discussion of Unlabeled/Investigational Use of Product/Device)
Natascha Pascale, MD - G58
Discloses no financial relationships with commercial entities.
Brittany D. Pasierb, BS - K30
Discloses no financial relationships with commercial entities.
Nisha V. Patel, BS - A26
Forensic Institute for Research and Education (Grant Support)
Anisha Paul, BS - A154
Agilent Technologies, Phenomenex (Discussion of Commercial Products or Services)
Lomiesha Paul - D58
Discloses no financial relationships with commercial entities.
Robert M. Pentz, BS - C10
The Aerospace Corporation (Discussion of Commercial Products or Services and Employee)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Applied Biosystems, Inc., Cybergenetics, Promega Corporation (Discussion of Commercial Products or Services) - A123
Cybergenetics (Employee) - A123, A125
Virginia Department of Forensic Science (Paid Consultant) - A125
Michel M. Perrier, MS - F22
Plass Data© (Discussion of Commercial Products or Services)
Donn N. Peterson, PE - E25
Discloses no financial relationships with commercial entities.
Nicholas Petraco, MS
Discloses no financial relationships with commercial entities - A142
Cargille Corporation (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device) - A189
Self Supported Research (Other Financial/Material Support) - A189
Anne C. Petty, JD - E14
Discloses no financial relationships with commercial entities.
Pierre-Antoine Peyron, MD - G118
Discloses no financial relationships with commercial entities.
Robert R. Pfalzgraf, MD - G167
Discloses no financial relationships with commercial entities.
Melinda Pham, BS - C4
J2 Scientific (Discussion of Commercial Products or Services)
Pennsylvania State College (Grant Support)
Abraham T. Philip, MD - G125
Discloses no financial relationships with commercial entities.
Ciaran F. Phillip, BS - A185
Coglan's, Coleman, Diamond, HomArt, Proforce, Redbird, REI, UCO (Discussion of Commercial Products or Services)
Virginia Commonwealth University (Grant Support)
Melissa Pickett - J8
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Texas A&M University (Other Financial/Material Support)
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Discloses no financial relationships with commercial entities.
Irene S. Pines, MA - D71
Discloses no financial relationships with commercial entities.
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Los Angeles County Inter-Agency Council on Child Abuse and Neglect (Grant Support)
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Discloses no financial relationships with commercial entities.
Haskell M. Pitluck, JD - E6, E48
Discloses no financial relationships with commercial entities.
Oscar A. Plaza, MD
Discloses no financial relationships with commercial entities - G57
ICITAP (Other Financial/Material Support) - G76
Amber Plemons, BS - H41
Discloses no financial relationships with commercial entities.
Cara A. Plese, MS - E24
Discloses no financial relationships with commercial entities.
Sharon L. Plotkin, MS - W16
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Isabelle Plu, MD, PhD - G35
Discloses no financial relationships with commercial entities.
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Bruker Corporation, Raving Dragon, Thermo Scientific, Shimadzu (Discussion of Commercial Products or Services)
National Institute on Drug Abuse (NIDA) (Grant Support)
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NLTK Project (Discussion of Commercial Products or Services)
Cristoforo Pomara, MD, PhD - G77, G128, G136
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Robert H. Powers, PhD - K56
Discloses no financial relationships with commercial entities.
Joseph A. Prahlow, MD - G74
Discloses no financial relationships with commercial entities.

Sebastien Prat, MD
Discloses no financial relationships with commercial entities - I12, I39
Société Pradel (Discussion of Commercial Products or Services) - G165
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Forensic Sciences Foundation (Grant Support)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Gary M. Pusillo, PhD - D87
Discloses no financial relationships with commercial entities.

Q

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R

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University of Tennessee (Discussion of Commercial Products or Services) - H36
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Praeger (Discussion of Commercial Products or Services)
Sumandeep Rana, MS - K57
HPLC System (Shimadzu) (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Jean-Sébastien Raul, MD, PhD - D43, K64
Discloses no financial relationships with commercial entities.
Simha E. Ravven, MD - I38
Twitter, YouTube, Facebook®, Inc. (Discussion of Commercial Products or Services)
Michelle H. Raxter, PhD - H122
Medical Metrics, Inc. (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device)
Harris County Institute of Forensic Sciences (Employee
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Synbone (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Eric J. Reichard, BS - A116
National Institute of Justice (Grant Support)
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Fox, Simon & Schuster, Inc. (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Fumedica AG, MAQUET Gmbh & Co., Scion Cardiovascular, Inc. (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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GE Global Research (Discussion of Commercial Products or Services)
Oak Ridge Institute for Science and Education (Speakers Bureau)
Golden G. Richard, PhD - B10
Microsoft Corporation (Discussion of Commercial Products or Services)
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EcoSmart Technologies, S.C. Johnson & Sons, Inc., Vertellus Specialties, Inc. (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Ambion, Applied Biosystems, Inc., Fisher, Norgen Biotek, Qiagen, Zymo Research (Discussion of Commercial Products or Services)
Federal Bureau of Investigation (Employee)
Rhonda K. Roby, PhD - A42
NicheVision (Discussion of Commercial Products or Services, Other Financial/Material Support, and Discussion of Unlabeled/Investigational Use of Product/Device)
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Zbot-Stealth (Discussion of Commercial Products or Services)
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Applied Biosystems (Discussion of Commercial Products or Services)
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Life Technologies, MicroLab Diagnostics, Promega Corporation, ZyGEM (Discussion of Commercial Products or Services)
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RTI International (Employee)
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Discloses no financial relationships with commercial entities.
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Toyota Motor Sales, U.S.A., Inc. (Discussion of Commercial Products or Services)
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National Institute of Justice (Grant Support)
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Khon Kaen University (Discussion of Commercial Products or Services)
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Access Data, Microsoft Corporation (Discussion of Commercial Products or Services)
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S

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Discloses no financial relationships with commercial entities.
Kelsey R. Sakaida - A32
Independent Forensics (Discussion of Commercial Products or Services)
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NIDA, NIH (Grant Support)
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Copan Spa (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities - G4
Wayne Rasband (Discussion of Commercial Products or Services) - G5
Eigenvector Research, Inc., Mathworks, ThermoNicolet (Discussion of Commercial Products or Services) - G54
Legal Medicine and Forensic Sciences in Portugal (Employee) - G55
Michael A. Sapienza, BS - C38
Shubees, Inc., Smithers Scientific Services, ASTM International (Discussion of Commercial Products or Services)
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Foster+Freeman (Discussion of Commercial Products or Services and Discussion of Unlabeled/Investigational Use of Product/Device)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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JPAC (Employee) - W10
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Forensic Sciences Foundation, Inc. Lucas Grant
Dick Hopkins/NecroSearch International Award (Grant Support)
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NetBio (Discussion of Commercial Products or Services, Employee, and Discussion of Unlabeled/Investigational Use of Product/Device)
Wolf Schweitzer, MD - G61
Siemens, Sectra Imtec AB, R Foundation for Statistical Computing (Discussion of Commercial Products or Services)
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AB SCIEX, Sanofi-Aventis, Shimadzu Corporation (Discussion of Commercial Products or Services)
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Texas State University (Employee)
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Discloses no financial relationships with commercial entities.
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Paternity Testing Corporation (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
Season E. Seferyn, MSFS - A60
National Institute of Justice (Grant Support)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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JEOL USA (Discussion of Commercial Products or Services)
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Canon, IBM, Konica Minolta (Discussion of Commercial Products or Services)
Wayne State University (Employee)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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SIMUL8 Corporation (Discussion of Commercial Products or Services)
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Immersion Corporation (Discussion of Commercial Products or Services)
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Life Technologies, ZyGEM Corporation, Ltd. (Discussion of Commercial Products or Services)
University of Virginia, Virginia Commonwealth University (Grant Support)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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School of Forensic and Investigative Sicnces, Universiity of Central Lancashire (UCLAN) (Employee)
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Southwest Institute of Forensic Science (Other Financial/Material Support)
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InnoGenomics Technologies, LLC (Employee)
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Discloses no financial relationships with commercial entities.
Edward Sisco, MS
Jeol USA (Discussion of Commercial Products or Services) - A140
Science, Mathematics, and Research for Transformation Scholarship (Grant Support) - A13, A140, A141
Katherine E. Skorpinski, MA - H92
Discloses no financial relationships with commercial entities.
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3D Systems, Creaform (Discussion of Commercial Products or Services)
Broward Sheriff's Office (Employee)
3D Systems (Discussion of Unlabeled/Investigational Use of Product/Device)
Brandi A. Skymba, MS - A204
Restek Corporation (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Mathworks, X-Ways (Discussion of Commercial Products or Services)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Richard R. Souviron, DDS - F31
Discloses no financial relationships with commercial entities.
Paul J. Speaker, PhD - W15
National Institute of Justice (Grant Support)
Kate Spradley, PhD - H27, H28
Discloses no financial relationships with commercial entities.
Jill L. Spriggs, MBA - E8, W8
Discloses no financial relationships with commercial entities.
Victoria A. Springer, MA - J9
National Institute of Justice (Grant Support)
Sargur Srihari, PhD - J7
Cedar (Discussion of Commercial Products or Services) - J7
National Institute of Justice (Grant Support) - J7, J10
Lisa Stadmeyer, MSFS - J4
FiberStone, Gartner, Inc., Natural Source Printing, Oxford, Terraskin, ViaStone Corporation (Discussion of Commercial Products or Services)
USPIS (Employee)
James E. Starrs, LLM - W21
Discloses no financial relationships with commercial entities.
Hilde Stawski, BS - A151
Illumina®, New England Biolabs®, Roche® (Discussion of Commercial Products or Services)
Carmen K. Steigman, MD - G115
Discloses no financial relationships with commercial entities.
Paul Stein, PhD - A6, D27
Discloses no financial relationships with commercial entities.
Jason R. Stenzel, PhD - K72
Washington State Patrol (Employee)
Carl N. Stephan, PhD - H123
ORISE (Grant Support)
Lashon Stephens, BS - A43
NicheVision Forensics, LLC (Discussion of Commercial Products or Services)
Peter R. Stephenson, PhD - B17
Discloses no financial relationships with commercial entities.
Peter R. Stephenson, PhD - B15
Facebook®, Inc. (Discussion of Commercial Products or Services)
Joyce K. Stocking, MS - G152
Chile Servicio Medico Legal (Employee)
Stephanie Stoiloff, MS - E8
Discloses no financial relationships with commercial entities.
Mark D. Stolorow, MS - E8
Discloses no financial relationships with commercial entities.
Peter R. Stout, PhD
Discloses no financial relationships with commercial entities - W12
Agilent Technologies, Applied Biosystems, Inc., Thermo Scientific, Waters Corporation (Discussion of Commercial Products or Services) - W19
RTI International (Employee) - W19
Briony C. Strachan, Msci - A78
Roche (Discussion of Commercial Products or Services), University of Virginia (Grant Support)
R.J. Straight, JD - B5
Kroll Advisory Solutions (Employee)
Selly R. Strauch, MD - G70
Discloses no financial relationships with commercial entities.
Phoebe R. Stubblefield, PhD - H22
University of North Dakota (Employee)
Kyra E. Stull, MS - H73
Lodox Systems, Ltd. (Discussion of Commercial Products or Services)
Andrew Sulner, JD - E55
Discloses no financial relationships with commercial entities.
Latha Sundar, PhD - C7
ARRO Forensics, Ltd. (Discussion of Commercial Products or Services)
Chasity D. Suttle, BS - K29
Forensic Institute of Research and Education at MTSU, National Science Foundation (Grant Support)
Yuvaneswari C. Swaran, PhD - A82
Applied Biosystems, Inc., Promega (Discussion of Commercial Products or Services)
Government of Malaysia (Other Financial/Material Support)
Kayla R. Sween, BS - A72
Promega (Discussion of Commercial Products or Services)
Cedar Crest College (Other Financial/Material Support)
Erin Sweeney, BS - A89
Discloses no financial relationships with commercial entities.
Madeleine J. Swortwood, BA - K73
Immunalysis, Neogen, OraSure, Randox (Discussion of Commercial Products or Services)
Immunalysis, National Institute of Justice, Neogen, OraSure (Grant Support)
Steven A. Symes, PhD - H31
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T

Silvia Tambuscio, MD
Altana Pharma, Bracco, Recordati (Discussion of Commercial Products or Services) - K67
Discloses no financial relationships with commercial entities - G28, G79
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Discloses no financial relationships with commercial entities.
Eugene Tan, PhD - A83, A84
NetBio (Discussion of Commercial Products or Services, Employee, and Discussion of Unlabeled/Investigational Use of Product/Device)
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Discloses no financial relationships with commercial entities.
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AB SCIEX (Discussion of Commercial Products or Services and Employee)
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JPAC-CIL (Employee)
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Applied Biosystems, Inc., Promega Corporation (Discussion of Commercial Products or Services)
National Institute of Justice (Grant Support)
Jayne E. Thatcher, PhD - W8
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Applied Biosystems, Inc., Promega Corporation, Qiagen (Discussion of Commercial Products or Services)
Lindsey C. Thomas, MD - E19
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Discloses no financial relationships with commercial entities.
Jennifer B. Thuor, MS - A158
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Federal Bureau of Investigation (Employee)
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Immersion Corporation (Discussion of Commercial Products or Services)
National Institute of Justice (Grant Support)
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Jessica K. Tokarz, BS - A62
Promega Corporation (Discussion of Commercial Products or Services)
National Institute of Justice (Grant Support)
Barbara Torres - J11
Los Angeles Sheriff's Department (Grant Support)
Pamela Tozzo, MD - G88
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Lauri Traub, JD - E11
Discloses no financial relationships with commercial entities.
Joe Trevino, BS - H91
ImageJ (Discussion of Commercial Products or Services)
Mihran Tuceryan, PhD - A198
National Institute of Justice (Grant Support)
James Tuckwiller - A25
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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National Institute of Justice (Grant Support)
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Florida International University (Employee)
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National Safety Council (Grant Support)
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Agilent Technologies, Phenomenex (Discussion of Commercial Products or Services)
NMS Labs (Employee)

U

Natalie Uhl, MS - H72
Discloses no financial relationships with commercial entities.
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Bruker Corporation (Discussion of Commercial Products or Services)
CNRS (Grant Support)
Joshuah M. Usilton
Smiths Detection (Discussion of Commercial Products or Services) - A2
West Virginia University (Grant Support) - A2, A3
Yuriy Uvaydov, MS - A212
Agilent Technologies, Cayman Chemical (Discussion of Commercial Products or Services)

V

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Discloses no financial relationships with commercial entities.
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Life Technologies (Discussion of Commercial Products or Services)
NIST (Employee)
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Kelly A. Walsh, PhD
Bureau of Justice Statistics (Grant Support) - A101
National Institute of Justice (Grant Support) - A219
Richard D. Walter, MA
Discloses no financial relationships with commercial entities - B17
Facebook®, Inc. (Discussion of Commercial Products or Services) - B15
Jeffrey P. Walterscheid, PhD - K78, W12
Discloses no financial relationships with commercial entities.
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Jane C. Wankmiller, MS - H121
Discloses no financial relationships with commercial entities.
Carley C. Ward, PhD - C34
Discloses no financial relationships with commercial entities.
Garth D. Warren, MD - G169
Bumbo International, Kidconnection, Summer SwaddleMe (Discussion of Commercial Products or Services)
Jodie A. Warren, MA - G151
Conviron Systems, Mathworks, PR Tools (Discussion of Commercial Products or Services)
Scott K. Watanabe, BS - A18
3M Incorporated, Duck™, Duncan Cr, Evercare™, Lyn-Peavey Co., Staples® (Discussion of Commercial Products or Services)
Daniel Watsula, MS
SAS (Discussion of Commercial Products or Services) - A152
Bode Technology (Employee) - A70, A152
Bode Technology, Promega Corporation (Discussion of Commercial Products or Services) - A70
Gabriel D. Watts, BA - J3
FLASH ID (Discussion of Unlabeled/Investigational Use of Product/Device)
Erin B. Waxenbaum, PhD - H49
Discloses no financial relationships with commercial entities.
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American Society of Forensic Odontology, Forensic Sciences Foundation, Inc. (Grant Support) - H66
Society of Forensic Odontology (Grant Support) - H65
Victor W. Weedn, MD, JD - G87
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Richard A. Weems, DMD - F8
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Crown Roll Leaf, Foster+Freeman, Keyence, Kubtec (Discussion of Commercial Products or Services)
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District 8 Medical Examiner's Office (Employee)
Daniel J. Wescott, PhD - H115
Texas State University (Employee)
Jessica L. Westland, MPS - A177
Penn State University (Grant Support)
Lauren K. Westling, BA - H119
Boston University (Other Financial/Material Support)
Eugene Wickenheiser, PhD - A105
Northern Michigan University (Employee)
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The Aerospace Corporation (Employee)
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Harris County Institute of Forensic Science (Employee)
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Vidacare (Discussion of Commercial Products or Services) - K38
City and County of San Francisco, International Federation of Professional and Technical Engineers - Local 21 (Employee) - K38, K41
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National Institute of Justice (Grant Support)
Ashley M. Wright - G44
West Virginia University (Other Financial/Material Support)
Diana M. Wright, PhD - A191
Federal Bureau of Investigation (Employee)
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X

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Qiagen (Discussion of Commercial Products or Services)

Y

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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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University of Tennessee (Other Financial/Material Support)
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Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Jorn C. Yu, PhD - G7
Sam Houston State University (Employee)
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R Core Development Team, University of Tennessee (Discussion of Commercial Products or Services)

Z

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Discloses no financial relationships with commercial entities.
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Acros Organics, Applied Biosystems, Inc., Dynarex, Invitrogen, Sigma Aldrich, Whatman International, Ltd. (Discussion of Commercial Products or Services)
National Institute of Justice (Grant Support)
Lauren Zephro, PhD - H67
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
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Bruker Elemental (Discussion of Commercial Products or Services)
Debra P. Zinni, PhD - H24
Discloses no financial relationships with commercial entities.
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Discloses no financial relationships with commercial entities.
Silvia Zoppis, MD - A46
Applied Biosystems, Inc., Promega Corporation (Discussion of Commercial Products or Services)
Brian C. Zubel, JD - E32
Applied Biosystems, Inc. (Discussion of Commercial Products or Services)
Torki Zughaibi, MS - A31
IonSense, Inc., JEOL USA (Discussion of Commercial Products or Services)
Saudi Arabian Cultural Mission (Other Financial/Material Support)

65th Anniversary

KEY WORD INDEX

1948 - 2013

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

65th Anniversary PRESENTING AUTHOR INDEX 1948 - 2013

The presenting author index can provide a quick reference to find when and in what section presenting authors are scheduled to present at the 2013 AAFS Anniversary 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	Criminalistics	J	Questioned Documents
B	Digital & Multimedia Sciences	K	Toxicology
C	Engineering Sciences	LW	Last Word Society
D	General	BS	Breakfast Seminar
E	Jurisprudence	ES	Evening Session
F	Odontology	L	Luncheon
G	Pathology/Biology	S	Special Session
H	Physical Anthropology	W	Workshop
I	Psychiatry & Behavioral Science		

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

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