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About the Editors

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Abstract

The 2016 National Institute of Justice (NIJ) Forensic Science Research and Development (R&D) Symposium is intended to promote collaboration and enhance knowledge transfer of NIJ-funded research. The NIJ R&D Program funds both basic or applied R&D projects that will (1) increase the body of knowledge to guide and inform forensic science policy and practice or (2) result in the production of useful materials, devices, systems, or methods that have the potential for forensic application. The intent of this program is to direct the findings of basic scientific research; research and development in broader scientific fields applicable to forensic science; and ongoing forensic science research toward the development of highly discriminating, accurate, reliable, cost-effective, and rapid methods for the identification, analysis, and interpretation of physical evidence for criminal justice purposes.

Introduction

On behalf of RTI International, the National Institute of Justice (NIJ), and the Forensic Technology Center of Excellence (FTCoE), we would like to present the proceedings from the 2016 NIJ Forensic Science Research and Development Symposium (R&DS). The Forensic Science R&DS was held February 23 in Las Vegas, Nevada, to promote collaboration and enhance knowledge transfer of NIJ-funded research. The NIJ Forensic Science Research and Development (R&D) Program funds both basic or applied R&D projects that will: (1) increase the body of knowledge to guide and inform forensic science policy and practice or (2) result in the production of useful materials, devices, systems, or methods that have the potential for forensic application. The intent of this program is to direct the findings of basic scientific research; research and development in broader scientific fields applicable to forensic science; and ongoing forensic science research toward the development of highly discriminating, accurate, reliable, cost-effective, and rapid methods for the identification, analysis, and interpretation of physical evidence for criminal justice purposes.

NIJ and the FTCoE are committed to improving the practice of forensic science and strengthening its impact through support of R&D, rigorous technology evaluation and adoption, effective knowledge transfer and education, and comprehensive dissemination of best practices and guidelines to agencies dedicated to combating crime. The future of forensic science and its impact on the public and criminal justice community is a motivating topic to gather expertise in a forum to discuss, discover, and share new research approaches and applications to promote the advancement of forensic sciences. The Forensic Science R&DS was specifically designed to bring together practitioners and researchers to enhance information-sharing with the goal of moving research from theory to practice. During this event, cutting-edge research was presented to an audience of over 350 attendees. The symposium included opening remarks from Gerald LaPorte, the Director of the Office of Investigative and Forensic Sciences, followed by four sessions that included 16 oral presentations. The symposium also included a presentation of the partnership between NIJ and the National Science Foundation (NSF), in which Rebecca Farrell from NSF gave an overview of how NIJ and NSF work together to advance fundamental research in forensic science. The Forensic Science R&DS was a unique forum that blended onsite participation with an online, interactive audience.

The preparation of the R&DS was greatly enhanced by the support and dedication of the NIJ R&D Program Management Team, which formed the steering committee who reviewed the abstracts; set the agenda; and developed a solid program of innovative scientific research. Our intent is that the 2016 R&DS proceedings will be a valuable and enduring resource to the forensic science and criminal justice communities.

IMPRESSION, PATTERN, AND TRACE EVIDENCE

MANTIS: Portable Prototype for Toolmark Research

IAA-2011-DN-R-0230

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A portable, prototype system designed to provide researchers and practicing examiners with a tool for conducting toolmark research studies has been developed. The system has the ability to obtain and characterize toolmark data in an objective, statistical manner. This program is a collaborative effort between industry and staff/faculty at Ames Laboratory/Iowa State University (AL/ISU). Design of the software interface was guided by consulting retired and current forensic examiners to develop a system that is intuitive, flexible, and easy to use. The Mark and Tool Inspection Suite (*MANTIS*) is based on an optical microscope and has the ability to measure surface topography quickly and accurately down to the submicron level. The device is small and can be packed in a hard-shell suitcase for transport. All acquisition and analysis software is resident on a laptop computer. Acquired data can be cleaned (if necessary) then the data files examined using the viewer built into *MANTIS* that mimics a comparison microscope in the ability to compare data files side by side. The operator has a wide range of sample movement, magnification, and rotation control and can activate a statistical algorithm that objectively determines the area of best fit for the marks, given operator-specified comparison parameters. A graph of the surface roughness of the marks is generated with the best-fit regions identified and the resultant statistical relevance is displayed. One feature of *MANTIS* is the ability to compare actual tools to toolmarks. Statistical comparison is possible since the system creates a "virtual mark" from the acquired tool data that can then be compared to the actual toolmark. Predictive capabilities as to the angle at which the evidence mark was made are possible by using a search routine built into the software and the embedded statistical algorithm. A blind study testing this ability identified 20 out of 20 screwdriver test marks, to within 5 degrees in most cases. Recent work using the system to examine knife cuts and ballistic markings have identified areas of improvement. These and other results will be presented and opportunities will be made available for examiners to test the system.

Keywords: toolmarks, statistical analysis, portable, *MANTIS*, predictive capabilities

ACEware: Standardizing ACE-V Documentation

2013-R2-CX-K011

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There is currently no widely used standard method of detailed documentation of the latent print examination process: how or whether examiners annotate what they use as the basis for their conclusions varies widely among agencies, and when examiners do document their work, interexaminer variation is extensive because there is no uniform training in feature-level documentation. Greater standardization of documentation is needed through more rigorous and consistent training and through tools for operational casework. ACEware seeks to address that problem by providing a platform for standards-based detailed annotation of the latent print examination process. ACEware is an innovative software tool for use in training new latent print examiners in standard, reproducible documentation of examination—as well as for use by experienced case-working latent print examiners in documenting actual casework. ACEware builds upon the FBI's Universal Latent Workstation (ULW), which allows users to create, edit, view, and manage latent fingerprint transactions. ACEware extends ULW by providing training functionality, extending its functionality for non-AFIS casework, and providing the capability to create standardized data sets for research and training. ACEware facilitates self-led and instructor-led examiner training and evaluation. Because ACEware documentation is based on the ANSI/NIST Extended Feature Set, detailed documentation of a complex latent print examination can be exchanged with other examiners, or archived for future review. ACEware is being developed by Noblis in collaboration and consultation with several federal, state, and local law enforcement partners.

Keywords: latent prints, fingerprints, ACE/V, training, documentation

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Using Oxygen and Strontium Isotopes to Dissect a Human Ecosystem: A Spatial and Temporal Study of Water and Human Hair from Southern California

2013-DN-BX-K009

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Critical advances in isotope mass spectrometry technologies and laboratory methodologies have allowed for increased application of oxygen ($^{18}O/^{16}O$) and strontium ($^{87}Sr/^{86}Sr$) isotope analysis of human hair to reconstruct an individual's travel histories. A first order assumption underlying these recent applications is that the $^{18}O/^{16}O$ and $^{87}Sr/^{86}Sr$ ratios of human hair are primarily influenced by isotope ratios of waters in that an individual imbibes (i.e., oxygen reflects regional precipitation) and bathes (reflects water interacting with strontium in bedrock). While these estimates are well suited for rural areas or municipalities exclusively using local water, these first order isotopic relationships may not be valid to model the isotope ratios of humans living in metropolitan areas that acquire their municipal waters from a mix of local sources (i.e., groundwater, surface waters) and distant regions (i.e., transported water). To characterize the temporal and spatial variability in the $^{18}O/^{16}O$ and $^{87}Sr/^{86}Sr$ ratios of water and associated human hair in megalopolis regions, we sampled Southern California metropolitan regions surrounding Los Angeles, Riverside, San Bernardino, and San Diego. These regions are ideal for a study of this type as they contain some of the largest, most densely populated metropolitan areas in United States and have well-known water-sourcing patterns. In these regions, we observed vast, but spatially distinct and coherent, oxygen isotopic variation in water. In addition, we noted temporal patterns in the oxygen isotope ratio of water in several municipalities, related to climate and seasonal transport. The strontium isotope ratios of waters in these regions also varied with water source. As hypothesized, we found the oxygen and strontium isotope ratios of hair samples collected in these regions largely reflected the isotope ratios of the water used within the individual municipality, suggesting that a municipality-scale approach is needed to model the isotope ratios of human hair in population centers.

Keywords: isoscapes, water management, urban, Los Angeles, San Diego

Forensic Biology/DNA

A Hybrid Machine Learning Approach for DNA Mixture Interpretation

2014-DN-BX-K029

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The challenge of DNA mixture interpretation is at the core of forensic genetic identification. Mastery of this interpretation can significantly impact the course of criminal investigations, the quality of intelligence, or both. In forensic settings, scientists responsible for mixture interpretations have relied on data from empirical validation studies, computation, and experience. Limitations are inherent in human-based analyses while other deficiencies are specifically related to computational capacity complexity and time constraints. There are expansive data sets that may be computationally leveraged to better address DNA mixture deconvolution. A classification approach capable of extracting maximum information from those data sets may be better able to interpret complex mixtures. A machine learning approach (MLA) is ideally suited to such complex data sets and can indeed be used in classification problems. Hybrid systems capable of combining human-like subjective reasoning with the computational power of artificial intelligence techniques may be of special interest. These MLAs may provide higher-confidence, more rapid, and expanded deconvolution capabilities. The power in such a system stems from (1) the ability of the algorithm to learn from an initial data set and subsequently classify mixtures from previously unseen data and (2) influence from the human analysts' experience-derived rule sets. The approach uses the strengths of both computationally intensive algorithms and expert systems. The architecture of the MLA permits mixture analyses using diverse data types including DNA fragment data, PCR amplification parameters and wide array of instrument parameters; this data agnostic structure will allow increased flexibility in adapting to analyses of new data types, such as next generation DNA sequence data. Candidate features were identified for extraction from mixture data sets such as peak height and ratios, and the subsequent evaluation will be the feature vector used as input for the machine learning algorithms. Several MLAs are being evaluated with training data drawn exclusively from mixtures of known contributors and proportions. The current MLA is able to evaluate mixtures of up to four contributors in less than 5 seconds and provide, with associated probabilities, (1) the number of contributors with a minimum accuracy of 90%, (2) the major component (minimum accuracy of 75%), and (3) deconvolute increasingly complex mixtures (minimum accuracy of 50%). This work is in progress and the fully optimized MLA is expected to outperform current benchmarks.

Keywords: DNA mixture, machine learning, forensic DNA, deconvolution, forensic biology

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Delivery of a Microfluidic Acoustic Sperm Cell Trapping Prototype for Rapid Processing of Sexual Assault Evidence

2013-NE-BX-K027

Forensic laboratories are often faced with sexual assault DNA evidence that requires labor-intensive, time-consuming sample processing, and streamlined workflows that deliver high-quality results with reduced cost and analyst time are needed. A microfluidic solution that exploits acoustic trapping offers an exciting paradigm shift from conventional differential extraction, one that can match the selectivity of current technologies while reducing the analysis time. Here, we propose to exploit the basic chemistry of current differential extraction (DE), but trap sperm cells specifically and directly using acoustics as a means of physically separating the male fraction (intact cells) from the female fraction (epithelial cell lysate) in evidentiary sexual assault samples. Acoustic-based microfluidic adaptation is an attractive option for this application as it combines high-throughput potential with low reagent and sample volume needs, a reduction in the number of pipetting and separation steps, and simplification of the protocol. Total time for Acoustic Differential Extraction (ADE) to go from swab to PCR-ready DNA is less than 1 hour. Initial NIJ funding allowed us to evolve from concept to successful demonstration of sperm cell trapping, and this was described in a 2009 publication. Using the application of acoustic energy in the 8–10 MHz range to the bottom of a microfluidic channel sets up a standing acoustic in two challenges remain to be addressed with the existing ADE system for in the fluid above it. In that standing acoustic wave, 1–3 nodes (depending on the dimensions of the channel) represent low-pressure zones where particles of a particular size, density, and compressibility are trapped in a monolayer, essentially suspended in the center of the channel. This represents the acoustic equivalent of optical tweezers. We exploit this to isolate intact sperm cells in a sample following lysis of the epithelial cells and, through control of fluid flow through the architecture of the microfluidic system, physically separate the sperm cell fraction from the female fraction. This allows for the male fraction with 100% male DNA (defined by qPCR) to be isolated from an overabundance of female epithelial cells. The instrument—SONIC-DE—that envelops and controls function of the acoustic microfluidic chip contains all of the necessary hardware for stable acoustic wave generation, microfluid flow control to and from six independent reservoirs, and a graphical user interface that allows the user to execute acoustic DE while visualizing the actual trapping event. Two have built two SONIC-DE systems and fabricated 70 acoustic trapping chips to be tested in two forensic laboratories on real samples.

Keywords: sperm cells, acoustic, trapping, microfluidic

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Chromatin-Based Sperm DNA Capture for Sexual Assault Samples

2014-DN-BX-K017

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Analyzing DNA from sexual assault samples presents unique challenges due to the presence of victim DNA, sample degradation, and trace amounts of DNA from the assailant. Conventional analysis of sexual assault samples relies on a process of differential extraction that is both laborious and time-sensitive; sperm heads must remain intact, which significantly limits the time window under which suitable sample can be collected from the victim. Alternative approaches to date have all required intact cells to allow isolation of the two DNA fractions. To circumvent the limitations of differential extraction, we have developed an alternative approach for isolation of sperm specific DNA from mixed samples based on affinity capture of proteins uniquely associated with sperm DNA. Chromatin-based sperm DNA capture takes advantage of the unique form of chromatin found exclusively within sperm heads. The DNA binding proteins protamine 1 (PRM1) and protamine 2 (PRM2) replace a majority of histones during the haploid phase of spermatogenesis and are absolutely specific to sperm chromatin. The PRM1:PRM2:DNA complex is incredibly stable in cell lysates, as we have learned when testing the dependence on fixation of the DNA: protamine complexes prior to capture, as is routine for less stable complexes typically analyzed by chromatin immunoprecipitation (ChIP). We have modified techniques used in traditional ChIP to target the DNA: protamine complex from solutions derived from mixed cells. As this process is dependent on a highly specific antibody, we undertook a screening of 10 antibodies using a modified Biacore assay to determine binding kinetics for the antibody to DNA: protamine complexes and have down-selected two candidate monoclonal antibodies and one polyclonal antibody that have shown the most promising results. The sensitivity of the capture was routinely demonstrated on as little as 100 input sperm, though the lower limit has yet to be completely defined. In mixed samples of sperm and epithelial cells, we routinely isolate sperm DNA with little carryover of epithelial DNA, and from samples with a 70:1 ratio of epithelial to sperm, clear short tandem repeat (STR) profiles were generated that identify the sperm. Preliminary results demonstrate the feasibility of our method which incorporates rapid sample lysis, novel antibody incubation conditions, and simplified sample purification in a workflow compatible with STR analysis. Adapting conventional ChIP protocols specifically for protamine-based sperm chromatin has enabled the streamlining of our method, while taking advantage of the strong DNA/protamine interaction. Chromatin-based sperm DNA capture has the potential to enable successful processing of sexual assault samples that are classically refractive to analysis, such as aged/lysed samples. Future experiments are focused on recovery of sperm specific DNA from semen-spiked swabs and vaginal swabs collected at extended intervals post-coitus. Method development is ongoing under NIJ award 2014 DN-BX-K017.

Keywords: sexual assault, rape kit, sperm, protamine

Robust STR Calling from High-Throughput Sequencing Technologies, Advanced Strategies for DNA Identification

2014-DN-BX-K089

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DNA profiles are a key investigative tool. In the past 25 years, US law enforcement agencies have developed an impressive array of protocols and databases to identify crime scene samples. Yet, the main working concepts of sample identification have largely remained the same. These include DNA amplification of CODIS/ENSFI markers (or short tandem repeats [STRs] on the Y-chromosome [Y-STRs]), genotyping STRs on a capillary platform, and querying law enforcement databases to find matches. In this talk, I will present several new strategies for sample identification. First, I will show a technique to recover surnames of individuals by analyzing Y-STRs and querying open databases for genetic genealogy. Second, I will discuss a new algorithm, called HipSTR, to genotype STRs from high-throughput sequencing data. The algorithm can recover missing STRs from nearby SNPs and report the actual sequence of the locus to refine a match. We used our algorithm to find 600 new Y-STRs and measure their mutation rates. Finally, I will discuss our work on human identification with a portable DNA sequencer by Oxford Nanopore MinION. I will present a novel strategy to identify samples using this device and present preliminary empirical results. The slides of the talk are publicly available at: http://bit.ly/FORENSIC

Keywords: forensic biology, Y-STR, DNA

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Measuring Rates of mtDNA Heteroplasmy Using a NextGen Sequencing Approach

2014-DN-BX-K022

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While current practice provides for reliable reporting of mitochondrial (mt) DNA haplotypes, the sequencing method in place today (Sanger) does not effectively identify heteroplasmic variants; especially low-level variants. Even when heteroplasmy is observed at high levels, the information is typically not used during a forensic investigation. The ability to detect, resolve and report heteroplasmy will significantly enhance the value of mtDNA analysis in forensic casework (Ivanov et al., Nature Genetics, 1996; 12:417–420). A next-generation DNA sequencing (NGS) approach will allow the community to achieve this goal, while maintaining the integrity of producing reliable haplotypes (Holland et al., Croatia Medical Journal, 2011; 52:299–313, McElhoe et al., Forensic Science International: Genetics, 2014; 13:20–29). The primary objective of our research was to measure the rate of mtDNA heteroplasmy in the mtDNA control region (CR) on a per-individual and nucleotide basis, in a European population group, and across different age groups; 550 samples from unrelated individuals. The NGS data was generated on a MiSeq from Illumina, Inc., and secondary data analysis was completed using the NextGENe® software from SoftGenetics, Inc. Depending on the threshold used, rates of heteroplasmy in the CR are relatively high (>70%), with >15% of the samples exhibiting heteroplasmy above 10%. The sites of heteroplasmy are asymmetrically distributed, with 6 out of 1122 positions accounting for >20% of heteroplasmic positions, and 959 sites where no heteroplasmy was observed. On the basis of our findings, we have begun to develop best practices regarding the reporting of mtDNA heteroplasmy in forensic cases, including the development of statistical models to address matching profiles and an assessment of the impact of error rates on the interpretation of low-level heteroplasmy. The results of this study relate directly to the theme of the 2016 American Academy of Forensic Sciences meeting, *Transformation: Embracing Change*, as this research is on the leading edge of a transformation in forensic DNA analysis. The use of an NGS approach to perform forensic mtDNA testing will vastly improve the discrimination potential of the method, and thus, will have a positive impact on society.

Keywords: forensic biology, next-generation sequencing, massive parallel sequencing, mtDNA, heteroplasmy, MiSeq

ANTHROPOLOGY AND MICROBIAL FORENSICS

The Transformation of Data Collection Procedures for Forensic Skeletal Material: Evaluating Osteometric Data in Forensic Anthropology

2013-DN-BX-K038

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Many forensic anthropology methods use osteometric data. Bone measurements are associated with objectivity and reliability compared to visual assessments of skeletal features. Osteometric data allow for the construction of confidence intervals, error terms, and probability estimates that lend credibility to these methods. However, osteometric methods are only as accurate as the measurements upon which they are based. This research investigates the reliability, accuracy, and validity of osteometric data. The results will be disseminated to the forensic community as a new digital and print edition of a widely used laboratory manual: *Data Collection Procedures for Forensic Skeletal Material 2.0.*

In 1985, Dr. Richard Jantz at the University of Tennessee obtained an NIJ grant (85-IJ-CX-0021) to standardize osteometric data recording procedures to facilitate data submission to a forensic anthropology data bank (FDB). The first edition of *Data Collection Procedures for Forensic Skeletal Material* (DCP) was published in 1988. This publication provided instructions for collecting data to be submitted to the FDB. By 1990, the FDB had 850 cases, and a software program was being developed to estimate sex and ancestry of unknown remains. Fordisc 1.0 revolutionized the manner in which forensic anthropologists construct biological profiles from skeletal remains. Since its inaugural release in 1993, Fordisc has relied upon osteometric data from the FDB, as specified by the data submission standards in DCP, last updated in 1994. Recent emphasis on reliable and tested forensic methods with known error rates has caused forensic anthropologists to question the reliability of certain measurements. In 2013, NIJ funded an effort to investigate error associated with osteometric data in DCP (2013-DN-BX-K038). The results of this research will be presented to the scientific community in 2016 as a new version of DCP (DCP 2.0).

DCP 2.0 will interface with the Fordisc software package, which will serve as the primary means of collecting skeletal and osteometric data for research and development purposes. The new manual omits problematic measurements, clarifies definitions, and introduces several new measurements. It also includes intra- and interobserver error rates and updated images. An accompanying video (in English and Spanish) demonstrates proper measurement techniques and helpful tips.

A comprehensive data collection effort was required to calculate intra- and interobserver error for each measurement. Four observers took 98 measurements on 50 skeletons. Each observer repeated the measurements four times with a 2-month lapse between sessions. Repeated measures analyses of variance with Tukey-Kramer post-hoc tests, technical error of measurement, and scaled error indices were used to assess measurement error. The results called for a number of modifications: (1) maximum and minimum midshaft diameters replaced all position-dependent measurements of shaft diameters (i.e.,

sagittal, transverse, dorso-volar, anterior-posterior); (2) pubis and ischium length were removed; (3) several measurements of epiphyses and articular surfaces were added (e.g., maximum olecranon breadth, maximum radial head diameter, AP diameter of S1, maximum glenoid cavity breadth); and (4) landmark and measurement definitions were refined to facilitate greater measurement precision. This transformation of DCP bolsters the foundations upon which forensic anthropology methods, research, and applications are constructed.

Keywords: anthropology, osteometric data, observer error, Fordisc

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A Multidisciplinary Validation Study of Nonhuman Animal Models for Human Decomposition Research

2013-DN-BX-K037

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Estimating the postmortem interval is an important component of missing person cases in the United States. Nonhuman animal cadavers, such as pigs and rabbits, are common proxies for human cadavers in decomposition research, yet methods and results from nonhuman studies have been extrapolated for use in criminal investigations without adequate validation to date. The purpose of this research is to apply both quantitative and qualitative techniques to assess the validity of two common proxies used in human decomposition studies: pig (*Sus scrofa*) and rabbit (*Oryctolagus cuniculus*) carcasses. We report our findings on the comparisons of decomposition patterns and scavenging among the three species across three trials in east Tennessee.

Five subjects of each species—human, pig, and rabbit—were placed on the ground surface in three seasonal trials at the Anthropology Research Facility at the University of Tennessee. All 15 rabbits were placed in cages to circumvent scavenging, while the pigs and humans were not caged. The total body scoring (TBS) method (Megyesi et al., J Forensic Sci, 2005; 50:618–626) was applied twice daily to help quantify morphological changes for each subject. Temperature data loggers recorded local temperature and humidity hourly. Motion-activated game cameras were placed in strategic locations (and moved when necessary) to capture images of scavenger activity.

The results to date indicate that the general decomposition pattern of pigs is more comparable to humans than that of rabbits. However, human decomposition is demonstrably more variable than either pig or rabbit decomposition, indicating that these nonhuman proxies do not account for the range of variation observed in humans. For instance, a fuzzy cluster analysis of the TBS scores showed that rabbits were an outlier to a more well-defined pig and human cluster. When rabbits were removed from the analysis, all of the pigs formed a tight cluster that also included some humans, while other humans were in a separate group. This reflects greater variability among the human subjects than the pigs. Differences in scavenging behavior were also noted between subject species. Raccoons preferred human remains over pig and rabbit subjects across all trials. Moreover, raccoons preferred some humans over others, although nearly all were scavenged to some degree. Pig scavenging was minimal and occurred only in Trial 3 after scavenging of the preferred humans was complete. Analysis of insect species and activities among the species is currently being analyzed and will be presented next year.

Keywords: forensic anthropology, taphonomy, nonhuman models, scavenging

Postmortem Changes and Translocation of Bacterial Community Structure and Function for Use in Criminal Investigations

2014-DN-BX-K008

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Commensal and transient microflora co-evolve and co-exist in complex, understudied networks on and within their host. The microbial community composition (e.g., diversity, abundance) can be specific to groups of individuals, sex, and anatomical area (e.g., gut, skin on the forearm). Recent evidence using animal model studies and the Human Microbiome Project have transformed the understanding of how microbes interact in a dynamic host and their overall impact on human and animal health. Specifically, it is now appreciated how commensal and pathogenic microbial communities influence host physiology (e.g., Crohn's disease) and behavior (e.g., schizophrenia, depression) in a variety of medically important ways. The activity and importance of the microbiome of a living individual, however, does not cease after death. Rather, at the time of death and shortly thereafter, the physiological changes resulting from decomposition can result in a proliferation in abundance and changes in species turnover (succession pattern) for the postmortem microbiome (PMM). This transition from ante- to postmortem microbial communities and the resulting succession of microbial species throughout decomposition may not only be an additional biological indicator of time since death, but these underexplored communities also have the potential to be used as trace evidence and for human identification purposes. Thus, scientists have begun to focus more on these microscopic organisms with the increased practicality and accessibility of next generation sequencing technologies for their potential application in a changing criminal justice system and evidentiary acceptability and interpretation.

This presentation encompasses the theme of the 2016 American Academy of Forensic Science Annual Meeting by providing an overview and data from NIJ-funded research investigating the changes in PMM structure and function and how these changes correlate to the transformation of a once-living host. Part one of this presentation will describe a novel series of controlled laboratory studies using fluorescently labeled bacteria in vertebrate models, imaging technology, bacterial culture, and molecular analyses to describe how the microbiome of a living host changes and transmigrates within the body after death. The second part of the presentation focuses on a collaborative effort to expand studies of the PMM by sampling a minimum of 75 human cadavers from six anatomical areas during routine death investigations at a major metropolitan medical examiner's office. This unique survey has maximized demographic representation by sampling individuals regardless of sex, age, race, or manner of death to establish a robust baseline data set of naturally occurring postmortem microbiota. Data presented provide a first step in linking the microbiome of a living being to the postmortem microbiome changes, which have demonstrated promise as evidence in criminal investigations. Overall, these studies support the potential of describing how microbial concentration, structure, and function affect the

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rate and overall process of decomposition, which has importance in forensic investigations and may lead to transformative forensic applications in the near future.

Keywords: postmortem, microbiome, transmigrate, decomposition

CONTROLLED SUBSTANCES AND TOXICOLOGY

Dried Blood Spot Analysis as an Emerging Technology for Application in Forensic Toxicology

2013-DN-BX-K017

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Hundreds of thousands of controlled substances and drugs of abuse are analyzed in forensic laboratories each year and are submitted as evidence in judicial cases. The abundance of drug-related cases can be a burden if laboratories are not able to analyze these samples in a timely manner. Dried blood spot (DBS) analysis is well-established in the area of clinical testing (e.g., screening of neonates for phenylketonuria) and, if adopted by forensic laboratories, has the potential to alleviate some of this burden. DBS has the potential to (1) increase sample stability, allowing samples to be stored for longer periods of time; (2) create a safer work environment for sample collectors and analysts because of the small sample volume requirement; and (3) significantly reduce costs in the area of transportation of DBS cards compared to traditional specimens. To date, we have evaluated 32 drugs and metabolites by DBS analysis using liquid chromatography–tandem mass spectrometry. Careful method development, including evaluation of card type, extraction solvent, spot volume, punch size, and internal standard addition method, was critical to determine optimum sample preparation conditions. We have determined that DBS analysis is sensitive enough for quantification of drugs of abuse (typical LOQs ranging from 2 ng/mL to 10 ng/mL with linear ranges up to 500 ng/mL for most drugs). Overall accuracy for 27 validated drugs and metabolites ranged from 86% to 112%. Between-run and within-run precision, measured as %CV, ranged from 4.6% to 9.7% and from 4.7% to 9.6%, respectively. Limit of detection ranged from 0.2 to 2.0 ng/mL. Sample extraction was performed with minimal solvent amount (e.g., 250 µL) and without further purification steps, eliminating the need for columns typically required for solid phase or supported liquid extractions. These results show that DBS analysis of drugs and metabolites in forensic laboratories produces results comparable to traditional drug analyses and has the potential to result in cost savings.

Keywords: dried blood spot, toxicology, liquid chromatography–tandem mass spectrometry

Characterization and Abuse of Electronic Cigarettes: The Efficacy of "Personal Vaporizers" as an Illicit Drug Delivery System

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Electronic cigarettes (e-cigarettes) have become popular to use as an alternative to traditional cigarettes, as a recreational activity, and as a delivery device for other licit or illicit drugs. They are filled with a variety of refill formulations (e-liquids) that typically comprise nicotine, water, propylene glycol (PG), and glycerin (VG). Many of these e liquids also contain a variety of flavoring and coloring agents, making them conceivably more appealing to a younger population of smokers. An analytical methodology to analyze the e-cigarettes, e-liquids, and the aerosol generated by the devices was developed to characterize the abuse potential in this growing industry.

Objectives

This research has had three main objectives:

- to identify information and resources to assist ongoing research to understand the forensic impact and implications of e-cigarettes,
- to develop a screening method using solid-phase microextraction (SPME) for analysis by gas chromatography–mass spectrometry (GC-MS) and Direct Analysis in Real Time AccuTOFTM Mass Spectrometry (DART-MS), and
- to characterize the volatiles produced in the aerosol of commercial e-liquids and to characterize e-cigarettes, e-liquids, and their aerosols by developing qualitative and quantitative methods of analysis by DART-AccuTOF^{TM} MS, high-performance liquid chromatography–tandem mass spectrometry, and GC-MS.

Results

- Google and IFTT searches revealed a robust community of experienced users describing and promulgating modifications and adulterations to e-cigarettes and e-liquids, including illicit drug delivery, through videos, social media, and user blogs, such as Reddit and YouTube. As of December 2015, 47 states have banned sales to minors, 8 have regulated the sales of e-liquids, and 7 have banned their use in public places. The media regularly reports on rising concerns of e-cigarette usage, ranging from concerns of second-hand exposure to exploding devices to methods of publicly consuming illicit substances.
- The SPME method using GC-MS and DART-MS detected several known constituents, including nicotine, propylene glycol, glycerin, and a variety of flavoring agents produced by e-liquids during aerosolization in an e-cigarette.
- 27 e-liquids with nicotine with labeled concentrations ranging from 0 to 22 mg/mL. They were found to contain nicotine ranging from 53 to 139 percent of the labeled concentration. The concentration of nicotine in the aerosols generated by the device increased as device voltage increased. An e-liquid sample purportedly containing THC and CBD confirmed positive for each.

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An effective method to screen and confirm e-liquids and an efficient mechanism for the capture and analysis of aerosols was developed to characterize the delivery of illicit substances and pharmaceuticals by e-cigarettes. As such, drug usage trends and abuse potentials can be more clearly defined for the criminal justice community.

Keywords: electronic cigarettes, e-liquids, vaping, drugs, SPME, DART-MS, liquid chromatography–tandem mass spectrometry

Should Forensic Laboratories Embrace Ultra-High Performance Supercritical Fluid Chromatography as a Separation Technique for the Analysis of Seized Drugs?

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The purpose of this project is to investigate the use of ultra-high performance (UHP) supercritical fluid (SFC) as a separation technique for forensic drug analysis, using the emerging drugs synthetic cannabinoids and bath salts as model compounds. These solutes, which can be very similar in structure, represent a difficult separation challenge. Determining the correct identity of these solutes is of utmost importance for the criminal justice system. The recently introduced separation technique, UHPSFC, produces highly efficient and rapid separations performed on a new generation of analytical SFC instruments with an environmentally friendly mobile phases containing primarily carbon dioxide, that have properties intermediate between a liquid and a gas. UHPSFC, like high-performance liquid chromatography (HPLC) and UHP liquid chromatography (LC), is advantageous for drugs that are thermally labile, polar, and nonvolatile solutes that are problematic for GC analysis. Compared to UHPLC, UHPSFC provides up to 4 times the separation speed. UHPSFC, which offers excellent selectivity for very similar compounds, is particularly useful for the separation of emerging drugs. The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), which is responsible for setting standards for drug analysis, does not list SFC as an approved separation technique. To be listed for SWGDRUG under Category B, as is the case for GC and LC, a separation technique should not only offer reasonable separation ability, but be orthogonal to accepted techniques such as GC and LC.

To ascertain whether forensic labs should adopt UHPSFC for forensic drug analysis, the separation of 33 synthetic cannabinoids (including positional isomers, diastereomers, and enantiomers) and the separation of 34 bath salts (including positional isomers and enantiomers) was investigated. In these studies, four achiral columns, including 2-PIC, Diol, DEA, and 1-AA (1.7 µm 3.0 x 100 mm), and three chiral columns, including AM1, CEL1, and CEL2 (2.5 µm 3.0 x 150 mm), were explored for the separation of synthetic cannabinoids and bath salts using carbon dioxide with various modifiers and additives. The modifiers included methanol, acetonitrile, ethanol, and isopropanol, while the additives included ammonium formate and ammonia. Detection was carried out by photo diode array–UV in series with single quad MS.

UHPSFC exhibited good resolving power for the separation of a mixture of controlled drugs, comparable to GC and UHPLC, taking into account the number of peaks resolved and the separation time. GC and UHPLC resolved more peaks with longer analysis time than UHPSFC. However, for one of the most difficult separations for GC and UHPLC, UHPSFC has been found to be far superior for the separation of JWH-018 and nine positional isomers. While at best, 4 out of 10 were resolved (resolution ≥1) by GC, all 10 were resolved by UHPSFC. Similar to GC and UHPLC, UHPSFC exhibited high resolving power for distinguishing between positional isomers of bath salts.

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The degree of orthogonality of UHPSFC, GC, and UHPLC was demonstrated for the separation of both synthetic cannabinoids and bath salts using principal component analysis.

Keywords: ultra-high performance liquid chromatography, synthetic cannabinoids, bath salts, seized drugs

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A New Approach to Drug Screening in Forensic Toxicology: Paper Spray Mass Spectrometry

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Current technology does not meet the need for rapid, effective, and simple drug screening methodologies in forensic toxicology. Our research seeks to develop a relatively new analytical technology called paper spray mass spectrometry (MS) into an effective tool for drug screening of postmortem blood samples and other forensically relevant specimens. In this method, drug detection by MS is carried out directly from a blood sample deposited on paper. It requires no sample preparation and can detect drugs and drug metabolites at forensically relevant levels (low ng/mL) directly from biofluid matrices. Other distinguishing characteristics of this technique include its simplicity, speed (1–2 minutes per sample), low blood sample volume consumed (~10 μL), and low solvent consumption (<100 μL per sample).

This project is evaluating and improving the capabilities of paper spray mass spectrometry for the rapid detection of 154 forensically important drug and drug metabolite targets for use as a drug screening method in toxicology. The major tasks are as follows:

- Determine the detection limits for the current state-of-the-art paper spray MS approach and improve where necessary.
- Evaluate the selectivity of the method by challenging it with isobaric compounds that could be encountered during forensic case work. Cross-interferences within the target compounds will also be assessed.
- Establish quality control mechanisms to improve robustness.
- Validate the screening assay in postmortem forensic toxicology samples, using confirmatory testing in collaboration with a practicing toxicology lab.

Forensic science is in a state of rapid evolution. Greater demands are being placed on forensic science laboratories, typically without a concomitant increase in financial resources. It is essential, therefore, that forensic science laboratories embrace new technologies that reduce costs and labor while improving, or at least maintaining, the quality of the data. Mass spectrometry is a powerful platform in the forensic laboratory for accomplishing this task.

Keywords: toxicology, paper spray mass spectrometry

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