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Abstract

The 2017 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 Forensic Science 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 2017 NIJ Forensic Science Research and Development Symposium (R&DS). The R&DS was held February 14 in New Orleans, LA, 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 sciences 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 300 attendees. The symposium included opening remarks from Gerald LaPorte, the Director, Office of Investigative and Forensic Sciences, followed by four sessions that included a total 16 of oral presentations. The 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 2017 R&DS proceedings will be a valuable and enduring resource to the forensic sciences and criminal justice communities.

IMPRESSION, PATTERN, AND TRACE EVIDENCE

The Fluid Dynamics of Droplet Impact on Inclined Surfaces with Application to Forensic Blood Spatter Analysis

2013-DN-BX-K003

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Bloodstain pattern analysis (BPA) is a technique used in crime-scene reconstruction to determine the point of origin of a blood droplet as well as its method of creation, e.g., dripping, wiping, or low-to-high-speed impact caused by anything from blunt trauma to cast-off to gunshot wounds. The primary problem of interest in BPA is to determine of the initial size, speed, and impact angle of a blood droplet that has struck a solid surface from an examination of the bloodstain pattern left on the surface. This problem was addressed in this work using an experimental study of the impact and spreading of a liquid droplet on planar surfaces of variable roughness, wettability, and absorbency oriented at various angles with respect to the velocity vector of the approaching droplet. The primary focus of this work is to use the shape of the final bloodstain to determine the initial conditions for the impact of the blood droplet.

Keywords: BPA, CSI, blood, droplet, bloodstain

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Illuminating Lifestyles by Metabolomics of Personal Objects

2015-DN-BX-K047

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Every individual has a unique lifestyle that results from personal habits. Our daily routines leave chemical traces on skin surface originating from the air we are exposed to, indoor environments; diet, exercise, clothes, medications, and our personal care products. In a scenario where these chemicals are transferred onto personal belongings that are routinely used such as phones, keys, purses, the chemistry left behind can reveal the lifestyle and habits of the individual who uses the artifacts. In this proof-of-principle study, we aimed to develop an untargeted mass spectrometry workflow to (1) characterize the chemistries recovered from personal objects of individuals and (2) evaluate the relationships between chemistries that can be found on objects and skin of the owners. Over 1,800 samples were collected from personal items (phone, keys, computer, wallet) and hands of 119 individuals, and further monitoring of phones and hands of 10 individuals was performed 4 months later. We used an untargeted high resolution UPLC-QTOF MS/MS-based workflow, combined to computational approaches to take a broad look at the chemistry of objects and hands samples. Molecular networking based on MS/MS spectral similarities was applied for a rapid characterization of chemical lifestyles recovered from personal items and to match molecular traces found on these objects to hands of individuals. We used a statistical pipeline to compare molecular profiles collected from objects and hands and to determine if molecular patterns from the skin can transfer to objects.

Our results reveal that chemicals recovered from personal objects are significantly unique to each individual and that these patterns are more similar to hands of the owners than to other individuals. Many molecular classes, revealed through molecular networking, were identified on objects ranging from beauty, hygiene products, diet, pesticides/insecticides, plasticizers, and medications such as antifungal, skin inflammation and anti-depressant treatments. Furthermore, we show that even molecules that were applied more than one month before sample collection can be detected on phones, such as diethyltoluamide (DEET), active ingredient of mosquito lotions. Some molecular traces tested 4 months later persist on phones and match the hands of same individuals sampled on time 1. Further, the combination of many identifiable chemicals detected on objects helps to predict the lifestyle of an individual. For example, detecting sunscreen derived molecules indicates that this person likely lives in a sunny area and spends time outside, molecules used as pigment dispersant in cosmetics suggest that the person is most likely a female using pigmented make-up, caffeine would indicate that the person is a coffee consumer, medicines will help to establish the medical status of an individual, while detection of citrus derived molecules indicate that oranges or lemons are part of personal diet. It is the combination of many such lifestyle routines that define unique chemical signatures.

These results introduce an additional form of trace evidence from skin-associated lifestyle chemicals found on personal belongings. Such information could help a criminal investigator narrowing down the owner of an object, found at a crime scene, such as a suspect or missing person.

Keywords: high resolution, UPLC-QTOF MS/MS, molecular profile, crime scene, CSI

Audio Forensics of Gunshot Sounds

2014-DN-BX-K034

Abstract: Gunshot acoustics—interpretation of the characteristic sounds produced by firearms recorded at a crime scene—is a specialization within the audio forensics field. Audio forensic evidence is increasingly common in law enforcement investigations because of the growing availability of inexpensive and lightweight digital voice recorders and miniature personal digital video camera systems for routine law enforcement and surveillance use. An increasing number of cases involving gunshot sounds are being captured in these audio recordings.

The acoustical characteristics of a firearm depend upon the type of gun and ammunition, the distance and azimuth with respect to the gun barrel, and the acoustical reflections and reverberation due to nearby surfaces and objects. For scientific study, it is necessary to separate the direct sound of the muzzle blast from the acoustic reflections, echoes, and reverberation that depend upon the recording environment. We use an elevated array of 12 specialized microphones capable of capturing the high intensity and short duration of the firearm's muzzle blast concurrently over 180 degrees in azimuth. Each microphone is recorded with 16-bit resolution at a 500 kHz sampling rate, and the elevated platform allows the entire muzzle blast to be recorded before the arrival of the first acoustical reflection from the ground.

This presentation includes a description of the firearm recording technique, the characteristics observed from these scientific recordings, recommendations on the use and processing of our database of firearm acoustical recordings, and a discussion of future prospects for forensic gunshot acoustical analysis.

Keywords: audio forensic, gunshot, acoustical analysis, firearms

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Characterization of Organic Firearms Discharge Residue: Progress and Potential

2011-DN-BX-K564

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The discharge of a firearm produces a wealth of physical and chemical evidence with unexploited evidential value. To date, forensic analysis focuses on characterization of particulates originating principally from the primer. These types of samples are collected on small metal stubs subsequently analyzed using scanning electron microscopy (SEM) coupled to X-ray spectroscopy (EDS). The method is described in ASTM method E1588. Several factors such as introduction of lead-free primers have contributed to an increased interest in characterizing the organic constituents of firearms discharge residue (OGSR). These residues, which consist of compounds such as diphenylamines, methyl and ethyl centralites, and dinitrotoluenes, offer an additional set of analytical targets that can be used to direct and supplement traditional methods of forensic analysis. However, it is critical in the early stages of this work that any proposed assay or method be based on instrumentation available to casework laboratories. Examples of such instrumentation include gas chromatography–mass spectroscopy (GC-MS) (used in seized drugs, toxicology, and trace evidence) and high performance liquid chromatography–mass spectrometry (HPLC-MS) variants used in forensic toxicology. For this research project, that has been a key consideration.

This presentation will summarize results to date from NIJ-sponsored research in this area related to characterization of OGSR on samples collected from the hands:

- Ion mobility spectrometry and chemometrics as a screening assay
- Simple direct thermal desorption-GC-MS to detect OGSR
- Sequential single swab characterization of organics and inorganics using GC-MS and inductively coupled plasma–mass spectroscopy (ICP-MS)
- Detection of elemental constituents of firearms discharge residue (GSR) using crown ether complexes and electrospray mass spectrometry
- Simultaneous detection of organic and inorganic GSR using flow-injection triple quadrupole mass spectrometry and crown ether complexes

Methodology and figures of merit will be presented along with results of samples obtained from the general public, known shooters, and known nonshooters, suggested pathways for adoption by the forensic, law enforcement, and legal communities. Advantages and limitations of each approach will be summarized along with suggested pathways for adoption by the forensic, law enforcement, and legal communities.

In addition, the results of a population study of ~70 individuals who provided questionnaires and anonymous samples will also be presented. These samples were subject to screening analysis using IMS to estimate the population background levels of several OGSR compounds.

Keywords: firearm, organic gunshot residue, OGSR, GSR, GC-MS, ICP-MS

Forensic Biology/DNA

Forensic DNA Phenotyping of Quantitative Pigment in Human Physical Appearance Prediction

2014-DN-BX-K031

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In order to circumvent the interpretation issue involved using categorical pigment descriptions in human physical appearance prediction (i.e., blue or brown eye color), understanding and predicting the phenotypic spectrum of quantitative color is key. This in combination with the advancement of technology and the ability to massively parallel sequence (MPS) DNA allows the potential to combine hundreds to thousands of markers that are needed to produce such a quantitative prediction result. Merging all currently known predictive pigmentation single nucleotide polymorphisms (SNPs) for eye, hair, and skin color into one biological platform, together with our quantitatively measured pigmentation prediction models, allows for a more precise prediction of some of the most obvious externally visible characteristics, pushing prediction towards a more individualized and unique pigment palette for investigators.

Keywords: DNA phenotyping, massive parallel sequencing, MPS, single nucleotide polymorphism, SNP

Proteomic Analysis of Menstrual Blood for Forensic Identification

2010-DN-BX-K192 and 2012-DN-BX-K044

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After attending this presentation, attendees will understand the general process of protein identification by mass spectrometry and how mass spectrometry–based proteomic analysis and statistical analysis of quantitative peptide data may be used to differentiate menstrual blood from circulating blood. This presentation will impact the forensic science community by illustrating the utility of proteomics for body fluid identification in forensic casework and, in particular, demonstrating the potential for an accurate, specific and confirmatory test for menstrual blood based on proteomic mass spectrometry. Differentiation of menstrual blood from circulating blood may play a key role in forensic investigations, particularly in cases of sexual assault. However, currently no confirmatory tests for menstrual blood are routinely being used in forensic casework. Menstrual blood is a complex body fluid composed of blood, endometrial, and immune cells shed from the uterus and vaginal fluid. The most abundant proteins in menstrual blood are shared with circulating blood, and proteins differentiating the two fluids are present in much lower abundance—making detection challenging. The goal of this work was to assess menstrual blood–specific protein markers in a large number of women of varying ages, ethnicities, and contraceptive use over the entire length of their periods.

Menstrual blood samples were obtained from 45 volunteers on each day of their periods, and one circulating blood sample was obtained from each volunteer during their periods. A total of 193 menstrual blood and 45 blood samples were collected, and the age, ethnicity, birth control method, if applicable, were recorded for each volunteer. Protein was extracted from all samples, and protein dynamic range was reduced using combinatorial peptide ligand chromatography in order to facilitate detection and identification of low abundance proteins. Samples were digested, barcoded with isobaric tags, and then combined. Peptides were separated by high performance liquid chromatography and analyzed by matrix-assisted laser desorption/ionization (MALDI) time of flight (TOF)/TOF mass spectrometry. A panel of candidate menstrual blood–specific markers proteins were identified which are inherent in uterine function and consistent with the biological processes of the menstrual cycle. These markers were present in all subjects regardless of ethnicity, age, or use of hormonal contraception, and consequently can be used to establish a robust and confirmatory assay. Additionally, because all peptides were labeled with isobaric tags, quantitative information about relative peptide abundance in each sample could be determined. Bioinformatics tools were used with quantitative peptide data to make a comprehensive comparison of patterns of protein expression in menstrual blood and circulating blood.

Keywords: menstrual blood, proteomics, mass spectrometry

An Optimized DNA Analysis Workflow for the Sampling, Extraction, and Concentration of DNA Obtained from Archived Latent Fingerprints

2014-DN-BX-K013

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DNA profiles have been obtained from fingerprints or fingerprint swabs, but there is limited knowledge about processing archived latent fingerprints where touch DNA is "sandwiched" between adhesive lift tape and paper. Scarce research on this sample type, along with the minimal success rates reported from touch DNA generally, often result in these samples being overlooked or dismissed as viable sources of biological evidence. In this work, 260 archived latent fingerprint samples from 15 volunteers were processed using a variety of methods in order to identify best practices for DNA workflow. From each volunteer, a set of archived latent fingerprints were either left untreated or subjected to visualization with black magnetic fingerprint powder or black carbon fingerprint powder. After disassembling, viable DNA was found on both the adhesive and paper side of the fingerprint sandwich. Further, taking direct cuttings of the disassembled archived latent fingerprint provided the highest DNA yields compared to those processed using either the single or double swabbing method (0.45ng vs. 0.12ng and 0.17ng, respectively). However, improvements using the double swabbing method were seen when the initial swab was pre-saturated with the lysis buffer/proteinase K (0.31 ng) compared to water (0.22 ng), Triton X-100 (0.16 ng), 91 percent isopropanol (0.12 ng), and 2 percent SDS (0.04 ng). Additionally, samples processed using the QIAGEN QIAamp® DNA Investigator Kit provided higher DNA yields and more detectable short tandem repeat (STR) allele peaks than samples processed using other methods (Invisorb® Kit, ZyGEM® Kit, and organic extraction). Further, use of one specific DNA concentration method, Centri-Sep™ columns, more than doubled the average number of STR loci detected. DNA profiles have been obtained from fingerprints or fingerprint swabs, but there is limited knowledge about processing archived latent fingerprints where touch DNA is sandwiched between adhesive lift tape and paper.

Keywords: DNA profile, latent prints

The Enhancement of the Native American CODIS STR Database for use in Forensic Casework

2014-DN-BX-K024

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Native American tribal DNA samples from the seven locations—which include the Arctic region, Baja California, California/Great Basin, the Southeast, Mexico, the Midwest, and the Southwest—were analyzed for allele frequencies, observed and expected heterozygosities, and F-statistics using the Globalfiler® PCR Amplification kit's 24 loci. Population-specific private alleles observed in this study may assist direct or indirect comparisons to identify the source of forensic evidence or infer tribal or ethnic origin. Geographic isolation and distance, as well as past migration events, have shaped and structured the population genetics of current day Native Americans in North America. The tribal samples exhibited an FST or θ value above the conservative 0.03 estimate recommended by the National Research Council (NRC) for calculating random match probabilities among Native Americans. This finding, together with lower levels of heterozygosity, implies the locations from which these samples were derived were both geographically isolated and also genetically subdivided. The greater differentiation among tribal populations (FST = 0.04) than had been previously estimated warrants the inclusion of additional regional Native American samples into short tandem repeat (STR) databases, such as Combined DNA Index System (CODIS).

Keywords: Native American, CODIS, STR, database

ANTHROPOLOGY AND MICROBIAL FORENSICS

Measuring Desiccation: A System Using Bioelectrical Impedance Analysis

2014-DN-BX-K015

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The estimation of postmortem interval (PMI) is a confounding factor in forensic investigation. The timing and chronology of an individual decomposition event may be accelerated or arrested by extant biotic and abiotic variables within a microenvironment, necessitating that an emphasis be placed on regionally specific models for PMI estimation. Current analytical models applied to late-stage PMI estimation are qualitative in nature and therefore are limited by individual experience and subjectivity. The most widely discussed method for quantitatively estimating PMI utilizes total body score (TBS) to estimate accumulated degree days (Megyesi, 2005). However, TBS relies upon qualitative categories of change assigned to an overly homogenous continuum, significantly reducing the objectivity of the method. This subjectivity is amplified in arid environments where rapid desiccation is followed by prolonged periods of stasis, slowing TBS while PMI continues to progress.

The western slope of Colorado is a high-altitude, semi-arid, cool climate desert region. Decomposition here is characterized by rapid desiccation. While gross changes are curtailed by periods of relative stasis, the hygroscopic nature of desiccated tissue results in dynamic patterns of adsorption of molecular constituents (such as lipid aggregation) and absorption of atmospheric moisture (facilitated by seasonally variable rain and snow fall) which continue to promote micro-morphological change. Concomitantly, the "typical" degeneration of cellular and soft tissue structures inherent to decomposition continue to progress. These parallel but distinct processes suggest the potential for qualitative and quantitative models to inform PMI estimation. In response to observed changes a dichotomous qualitative/quantitative model for estimating PMI was developed and is currently being tested among a sample of human donors at the Colorado Mesa University Forensic Investigation Station (FIRS).

Breakdown of biological tissue is a universal feature of decomposition. Bioelectrical impedance analysis (BIA) is currently being tested as a quantitative means for measuring this process of degradation. Because BIA technology was originally developed as a means for quantifying the proximate body composition (water, lipid, and lipid-free dry masses) of living humans, it is uniquely suited to decomposition studies. BIA technology measures the resistance and capacitive reactance of an electrical current passed through biological tissue. Within biological tissue the intra- and extra-cellular fluid act as resistors, whereas cell membranes act as capacitors. Decomposition results in changes in the structure of these biological resistors and capacitors that can be quantified using BIA techniques.

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Observations from 3 years of study at FIRS informed a qualitative scoring system—termed total body desiccation score (TBDS)—to increase the resolution of gross changes presented by desiccated remains. Changes in color, tissue quality, release and integration of moisture, and the thickness of tissue layers are some of the specifics noted to date. The authors hypothesize that these changes correlate to ranges of accumulated degree days and therefore may be used to refine methods for estimating PMI.

A study was launched in late 2015 using both methods (BIA and TBDS) demonstrating significant correlations between postmortem interval and accumulated degree days (ADD). Highlights of the progress to date are presented, as well as future directions.

References

Megyesi, M., Nawrocki, S. P., & Haskell, N. H. (2005). Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *Journal of Forensic Science*, *5*(3), 618–626.

Keywords: postmortem interval (PMI), total body desiccation score (TBDS), bioelectrical impedance analysis (BIA)

Statistical Methods for Combining Multivariate and Categorical Data in Postmortem Interval Estimation

2013-DN-BX-K042

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To our knowledge, an estimate of time since death is almost never accompanied by the kind of mathematically explicit probability statement that is the standard in most scientific disciplines. This has been a problem both for death investigation casework (and court testimony) and for research, because scientists have not known how to design decomposition experiments to provide adequate statistical power for postmortem interval (PMI) estimation. We have been developing methods for calculating statistical confidence limits about a PMI estimate based on either continuous quantitative or categorical data. The examples we present are from forensic entomology, but the approach is suitable for any postmortem variable.

To do this we extended and adapted the time-tested statistical method of inverse prediction (IP; also called calibration) to the PMI estimation setting. Methods to produce valid p-values for this process are known for single, quantitative y and x that follow a linear regression relation and with y having constant variance. Some exist for multivariate y, but only for settings where y has constant variance. Many measurements used for PMI estimation do not fit these criteria. The current project builds on earlier work in which we developed IP methods for nonconstant variance of a single, quantitative y (e.g., estimating carrion maggot age using a single size measurement); and in which we developed the first ever method for IP based on categorical data (e.g., estimating PMI based on carrion insect succession).

One possible barrier to the adoption of these new inverse prediction methods by researchers and death investigators has been that they are not implemented in statistical software packages. In this presentation, we will show how IP using categorical data can be done by simply reading a table. Concerning quantitative data, we will show how inverse prediction of PMI can be performed using statistical analysis software already widely available for general linear mixed models, where the statistical theory and methodology are well-established. We will show how flexible models using polynomial splines can be fit for both the means and variance-covariance matrices, and how dummy variables can be used over a grid of values of x to get the p-values required for confidence sets automatically. Attendees familiar with mixed models and their applications will be able to implement these methods in standard statistical packages.

Keywords: postmortem interval estimation, inverse prediction, calibration, multivariate statistical methods

The Isotopic Taphonomy of Human Hair

2014-DN-BX-K002

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After attending this presentation, attendees will understand the suitability of hair samples for stable isotope analysis (SIA) in samples from cadavers decomposing in two outdoor sites. This study will impact the forensic science community by increasing our knowledge of the strengths and limitations of this type of analysis for identification of unknown human remains. It will also promote the benefits of SIA to the law enforcement community.

Isotope ratios in human remains can record diet, birthplace, and residential history—useful information for identifying unknown individuals. They have proved highly constructive in forensic cases reported in the literature, and are being implemented in an expanding number of academic and research labs across the country and internationally. However, no systematic study has evaluated how decomposition and outdoor exposure may modify measured ratios from their premortem values.

The purpose of this study is to evaluate the fidelity of different isotope systems in human hair, teeth, and bone through the processes of decomposition. To accomplish this, we measured light stable isotopes (carbon, nitrogen, hydrogen, and oxygen), radiogenic isotope ratios (strontium and lead), and trace elements in tooth enamel, bone, and hair from seven individuals from body donation programs. The donors were placed on the ground surface at the anthropological research facilities at the University of Tennessee, Knoxville and Texas State, San Marcos. These two sites were selected to provide a range of rainfall and temperature conditions as well as different underlying geologic lithologies.

Rainfall samples, well water (Texas), groundwater (Tennessee), and soil samples were collected and analyzed for the same suite of isotopic and elemental parameters. The goal of the environmental materials that might be exchanging or mixing with the cadaver samples. Hair collected up to 106 days was mechanically and chemically cleaned, homogenized, and processed using standard protocols. There were no systematic variations in carbon, nitrogen, oxygen, and hydrogen isotope ratios over this time. In addition, we measured hair mats from 10 individuals placed on the surface at Texas State, and compared them to intake samples, and found no variations in carbon, nitrogen, oxygen or hydrogen isotopes for up to 312 days.

However, strontium isotopes of bulk hair in the seven individuals are modified in the direction of bioavailable strontium from local soils over time. In addition, some trace elements (Mn, Co, Mo, rare earth elements, and U) demonstrate increases over time. Bone and tooth enamel samples were also collected and are in the process of being analyzed.

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It appears that light stable isotopes (carbon, nitrogen, oxygen, and hydrogen) in human hair preserve pre-mortem values in outdoor placements on the ground surface in several environments over at least 4 months. The values of strontium and lead isotopes have a more complicated behavior that further research may be able to elucidate.

Keywords: stable isotope analysis (SIA), taphonomy, human hair

Adult Skeletal Age Estimation: Tackling Long-Standing Problems with a New Approach

2014-DN-BX-K007

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Accurate and precise age estimates are critical for identifying people represented by skeletal remains. Unfortunately, useful age estimates for adults are beyond what standard methods can provide. Existing methods yield biased point estimates of age accompanied by ranges that usually span several decades to virtually all of adulthood. Age intervals assigned to skeletons often cannot narrow the search to certain categories of missing people or provide support for individual identifications. Furthermore, little can be said with confidence about the age of people beyond about 50 years.

This NIJ project (2014-DN-BK-K007) involves (1) defining new skeletal traits, (2) assessing observer error, (3) establishing trait age distributions, (4) modifying existing mathematical procedures, and (5) developing a user-friendly computer program to estimate age. These tasks must be undertaken in one project to make the best use of age-related skeletal variation, accommodate long-recognized analytical issues, and ensure the wide acceptance of a computationally intensive procedure.

The new procedure is an improvement on an existing approach to age estimation, referred to as Transition Analysis, which previously focused solely on the pelvic joints and cranial sutures. The NIJ project relies on multiple traits distributed throughout the skeleton. Each trait undergoes a transition from one state to the next during adulthood, as estimated from many known-age individuals. Those transitions give the probability of being in a particular state at each age; the probabilities are then combined to yield an age estimate. A computer program being developed as part of the project enables forensic practitioners to take advantage of the age-estimation procedure.

Data have been collected for 79 traits from over 1,600 known-age adult skeletons. A large sample from four continents captures regional and ancestry-related variation in the aging process. Many traits, such as those in the humerus and femur, have previously seen little attention. Traits are usually scored as binary states (absent-present). Readily visible changes throughout the skeleton collectively provide an inexpensive, fast, and nondestructive means of estimating age. Because the procedure relies on bony features distributed throughout the body, age estimates are possible for incomplete skeletons, which is essential for forensic investigations.

We present examples of useful skeletal age indicators and associated ages of transition. Ages at transition are distributed throughout adulthood, enabling estimates to the maximum human lifespan, an impossibility with existing methods. Numerous skeletal features analyzed in a transition analysis framework yield far better age estimates throughout adulthood than either existing transition analysis (pelvic joints and cranial sutures alone) or other commonly used methods. Results to date demonstrate that improvements in age estimation will only come about through sophisticated statistical procedures coupled with new age-informative skeletal traits.

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In short, a much-improved adult age-estimation procedure is being produced. This work shows that anthropologists should shift their attention to many traits distributed throughout the skeleton, rather than continue their century-long focus on only a few age-informative features, principally the cranium and pelvis.

Keywords: skeletal, age estimation, transitional analysis framework

CONTROLLED SUBSTANCES AND TOXICOLOGY

Novel Blood Protein Modification Assay for Retrospective Detection of Drug Exposure

2015-NE-BX-K001

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Detection and confirmation of human exposure to drugs typically relies on measurement of parent compounds or metabolites in blood, urine, or an alternative sample matrix. This "biomonitoring" approach is widely employed for forensic toxicological applications. However, such data generally cannot provide insight into past episodic exposure, cumulative exposure, or time-dependent exposure profiles. Nevertheless, such information may be critically important in, for example, suspected drug facilitated crime and assessment of drug compliance or abstinence.

Currently, retrospective biomonitoring of drug use or exposure is limited to analysis of hair, for which standardized methods and a large database exists. While clearly useful for this purpose, hair analysis does have technical and interpretive drawbacks. Another potential technology for longer-term monitoring of drug exposure involves measurement of the products of covalent modification of thiol moieties of blood proteins, such as hemoglobin (Hb) and serum albumin (SA), by reactive metabolites (RM) of drugs. Since they typically persist for the life of the protein, such "adducts" can provide a much longer window of detection of exposure than is possible by direct measurement of parent compound or a metabolite. While widely used in human exposure assessment for environmental and occupational chemicals, applications of protein adducts as markers of illicit drug exposure are virtually nonexistent.

This project is establishing proof-of-concept for development of a robust protein adduct detection technology for drugs of abuse. Initial work involved optimization of an in vitro assay system to generate RM of drugs of abuse with the inclusion of glutathione (GSH) or an N-acetylated thiol-containing model peptide (Ac-PAACAA) as trapping agents to characterize (via liquid chromatography triple quadrupole mass spectrometer (LC-QqQ-MS) and liquid chromatography quadrupole time of flight mass spectrometer (LC-QTOF-MS) covalent adduct structure and stability. Selected compounds included 16 licit and illicit drugs from a variety of structural and pharmacological classes. In these experiments, GSH adducts of 10 of the 16 test drugs were identified via LC-QqQ-MS fragmentation patterns common to GSH adducts. These data were then confirmed and adducts structurally characterized using high-resolution mass spectrometry(HRMS). We report for the first time the formation of covalent GSH adducts of diazepam, 3,4-methylenedioxypyrovalerone (MDPV), naltrexone, oxycodone, and Δ9-THC, in addition to confirming previously reported data for cocaine, morphine, MDMA, acetaminophen, and clozapine. Covalent adduction to the Ac-PAACAA model peptide was observed for all 16 of the test compounds, with numerous drugs showing high potential for adduct formation. Mass spectrometer/mass spectrometer (MS/MS) sequencing data confirmed the identity of the major peak for each drug as a drug-peptide adduct located at the thiol moiety.

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Demonstration of the capability of commonly abused drugs to covalently bind to thiol residues in vitro represents a critical step in assessing their protein binding capabilities for assay development. Ongoing work is focused on detection and characterization of thiol modifications of human Hb and SA by RM of the selected drugs following in vitro incubation of purified human proteins and human whole blood. A final goal is to confirm protein adduction and validate adduct detection protocols in a set of authentic blood specimens from subjects enrolled in addiction treatment and other drug screening programs.

Keywords: drug exposure detection, high resolution mass spectrometry, HRMS, LC-QTOF-MS

Stability of Synthetic Cathinones in Biological Evidence

2013-R2-CX-K006

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The ongoing proliferation of designer drugs present a variety of public health and safety concerns. Synthetic cathinones are capable of producing a variety of psychostimulant effects. According to the National Forensic Laboratory Information System (NFLIS), their use has escalated. Forensic laboratories must be able to identify these new drugs as part of antemortem and postmortem toxicology investigations. Due to limitations in immunoassay-based screening technologies, many forensic toxicology laboratories must rely on chromatographic-based screening approaches in order to detect these drugs in biological evidence.

The detection of drugs is heavily dependent upon the stability of the drug in biological matrices, information that is relatively limited for synthetic cathinones. This research presents a validated method for the quantification of 22 synthetic cathinones in urine and blood using liquid chromatography/quadrupole time of flight mass spectrometry (LC/Q-TOF-MS). The validated method was used to systematically evaluate the stability of synthetic cathinones in blood and urine over a six-month period. Drug stability was assessed in terms of pH, temperature, matrix, concentration-dependence and chemical properties. Solid phase extraction (CEREX Polycrom Clin II) and LC/Q-TOF-MS (Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS) equipped with a Poroshell 120 EC-C18 column were used to detect 22 synthetic cathinones in urine and blood. Specimens were evaluated on the order of hours, days and weeks, depending on the rate of degradation.

The following cathinones were included in the study: methcathinone, ethcathinone, pentedrone, buphedrone, 3-fluoromethcathinone (3-FMC), 4-fluoromethcathinone (4 FMC), 4-methylethcathinone (4-MEC), 4-ethylmethcathinone (4-EMC), mephedrone, methedrone, 3,4-dimethylmethcathinone (3,4-DMMC), ethylone, butylone, pentylone, eutylone, methylone, methylenedioxypyrovalerone (MDPV), 4 methylpyrrolidinobutiophenone (MPBP), 3,4-methylenedioxypyrrolidinobutiophenone (MDPBP), alpha-pyrrolidinopentiphenone (alpha-PVP), pyrovalerone, and naphyrone.

Nine deuterated internal standards were used. The analytical method was validated in terms of limit of detection and quantitation, precision, bias, interferences, matrix effect, dilution integrity, analytical recovery, carryover, and calibration model. Limits of detection and quantitation ranged from 0.25 to 5 ng/mL for urine and 1 to 5 ng/mL for blood. In urine, accuracy ranged from 97 to 112 percent and intra/interassay precision ranged from 0 to 11 percent and 2 to 12 percent, respectively. In blood, accuracy ranged from 94 to 111 percent and intra/interassay precision ranged from 0 to 9 percent and 3 to 7 percent, respectively.

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Cathinone stability was evaluated at high (1000 ng/mL) and low (100 ng/mL) concentrations, at variable urinary pH (pH 4 and 8), in two matrices (blood and urine), and at four temperatures (-20°C, 4°C, 20°C, and 32°C). Cathinones were considered stable if concentrations were within 20 percent of the expected value. In both matrices, the pyrrolidine-methylenedioxy type (MDPBP and MDPV) synthetic cathinones were the most stable. Cathinones with benzylic substituents were among the least stable. Under certain conditions of storage, some cathinones were completely degraded within hours. However, all cathinones were stable under frozen conditions. Degradation was highly pH dependent, exhibiting increasing stability with decreasing (more acidic) pH. Although pH, temperature and chemical structure played an important role, no concentration dependence was observed. A greater understanding of cathinone stability in biological evidence will improve the interpretation of antemortem and postmortem toxicology results in forensic investigations.

Keywords: toxicology, synthetic cathinones, stability

Towards Development of a Mass Spectrometric Database for Rapid Identification of Plant Drugs of Abuse Using Ambient Ionization Mass Spectrometry

2015-DN-BX-K057

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With increasing frequency, the drugs being submitted to crime labs are no longer restricted to well-characterized substances such as cocaine, heroin, and prescription drugs, among many others. The use of new psychoactive substances (NPS), especially plant-based psychotropics, has increased due to the marketing of these products as safe and "legal" alternatives to common drugs of abuse. The use of plant-based drugs of abuse quadrupled between 2009 and 2013 and now accounts for nearly 10 percent of NPS on the drug market. Currently, there are few standard operating protocols (SOPs) available to characterize and identify these complex plant matrices. Furthermore, the constantly changing landscape in the variety of abused plant products makes method development and validation for each new drug unrealistic, particularly when relying on conventional methodologies. An added dimension to the problem of identifying psychoactive plant products is the lack of statistical analysis in forensic science reporting, as highlighted in the 2009 National Academy of Sciences report. While statistical analysis could be performed on data generated by gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS), the number of replicates needed and the time required to process individual samples makes this approach highly impractical. These challenges are further exacerbated by the absence of SOPs that are standardized across local, state and federal crime labs.

Direct analysis in real time–high resolution mass spectrometry (DART-HRMS) provides an opportunity to circumvent some of the disadvantages of current drug testing methodologies, and the utility of the method is demonstrated here. Toward the creation of an abused plant database, plant material purported to be psychoactive was tested directly without the need for complex sample preparation steps such as extraction, pH adjustment, and derivatization, to obtain a chemical fingerprint of the species. Datura spp. seeds, kratom powder, kava powder, Salvia divinorum leaves, kanna crushed leaf material, Amanita muscaria mushroom caps, and morning glory seeds were analyzed in their native forms using DART-HRMS under soft ionization conditions. The psychoactive substances contained within the complex matrices of the plant-based psychotropics were identified from accurate mass information and elemental composition determination. Identified compounds include mitragynine in kratom, atropine and scopolamine in Datura spp., salvinorin A in S. divinorum, and four individual kavalactones in kava. The plant materials were subjected to in-source collision-induced dissociation to confirm the structural assignments of the psychotropic compounds in the cases where authentic standards were available.

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The complete mass spectral profile of the psychotropic plant materials provided a diagnostic chemical fingerprint which was unique enough to enable species identification and classification through the application of multivariate statistical analysis methods, including linear discriminant analysis (LDA), hierarchical clustering analysis (HCA), and partial least squares discriminant analysis (PLSDA). The application of statistical analysis methodologies enabled species-level identification of plant-based drugs of abuse with a defined level of confidence. The results serve as a foundation upon which a database of abused plants can be created to aid in their rapid identification in a forensics context.

Keywords: mass spectrometry, psychoactive substances, plant material, database, kratom

One Pot Methamphetamine Effluent Characterization

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The One Pot methamphetamine production method has become the primary method of choice in clandestine drug laboratories across the United States, due to its simplicity and the availability of required materials. While the method is simple, it also generates risk to innocent bystanders within the community from flammability and toxicity hazards. This study was undertaken to determine the feasibility of the detection of clandestine methamphetamine laboratories through monitoring waste water effluents. Methamphetamine was produced by the One Pot method and the methamphetamine hydrochloride product was filtered out. The remaining materials were deposited into a local waste water system in a controlled setting to simulate the disposal of unwanted production products. Water samples were collected post-distribution to determine a time course and analyzed via solid phase extraction with liquid chromatograph-tandem mass spectrometry. Methamphetamine, pseudoephedrine, and ephedrine were all detectable in the waste water. Also, an over-reduced product characteristic of the One Pot synthesis, CMP [1-(1',4'-Cyclohexadienyl)-2-methyl-aminopropane] was detected. This work demonstrates the possibility and potential for analyzing waste water to monitor and detect clandestine One Pot methamphetamine laboratories within a community.

Keywords: clandestine laboratory, One Pot, methamphetamine

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