RTI Press

Conference Proceedings

February 2019

2019 National Institute of Justice Forensic Science Research and Development Symposium

Nicole S. Jones and Erica Fornaro, Editors

RTI INTERNATIONAL

RTI Press publication CP-0009-1902

RTI International is an independent, nonprofit research organization dedicated to improving the human condition by turning knowledge into practice. The RTI Press mission is to disseminate information about RTI research, analytic tools, and technical expertise to a national and international audience. RTI Press publications are peer-reviewed by at least two independent substantive experts and one or more Press editors.

Suggested Citation

Jones, N. S., and Fornaro, E. (Eds.). (2019). *2019 National Institute of Justice Forensic Science Research and Development Symposium*. RTI Press Publication No. CP-0009-1902. https://doi.org/10.3768/rtipress.2018.cp.0009.1902

This publication is part of the RTI Press Conference Proceedings series.

RTI International
3040 East Cornwallis Road
PO Box 12194
Research Triangle Park, NC
27709-2194 USA

Tel: +1.919.541.6000
E-mail: rtipress@rti.org
Website: www.rti.org

This forum was funded through a cooperative agreement from the National Institute of Justice (2016-MU-BX-K110), Office of Justice Programs, US Department of Justice. Neither the US Department of Justice nor any of its components operate, control, are responsible for, or necessarily endorse, this forum. The opinions, findings, and conclusions or recommendations expressed in this forum are those of the presenter(s) and do not necessarily reflect those of the Department of Justice. Any products and manufacturers discussed are presented for informational purposes only and do not constitute product approval or endorsement by the US Department of Justice.

©2019 RTI International. RTI International is a registered trademark and a trade name of Research Triangle Institute. The RTI logo is a registered trademark of Research Triangle Institute.

This work is distributed under the terms of a Creative Commons AttributionNonCommercial-NoDerivatives 4.0 license (CC BY-NC-ND), a copy of which is available at https://creativecommons.org/licenses/by-nc-nd/4.0/legalcode

Contents

About the Editors i
Steering Committee iii
Planning Committee iii
Project Directors iii

ABSTRACT iii

INTRODUCTION iv

IMPRESSION AND PATTERN EVIDENCE/TRACE EVIDENCE 1

Quantitative Assessment of Shoeprint Accidental Patterns with Implications Regarding Similarity, Frequency, and Chance Association of Features—Empirical Chance Association of Randomly Acquired Characteristics 2
Jacqueline Speir

Finding the Region of Origin of Bloodspatters in Complex Situations: Physical Description, New Data, Tools, and Reconstruction Method 3
Daniel Attinger

Raman Microspectroscopy and Advanced Statistics for Detection and Characterization of Gunshot Residue 4
Igor K. Lednev

Consistent Single Shot Detection of Organic and Inorganic Residues from One Sample using LC/MS and Host-Guest Complexes 6
Suzanne Bell

FORENSIC BIOLOGY/DNA 7

A Method to Estimate the Age of Bloodstains Using Quantitative PCR 8
Robert W. Allen

Proteomic Analysis of a Single Human Hair for Ancestral Classification—Use of Genetically Variant Peptides to Statistically Estimate the Genetic Background of Hair Shafts 9
Zachary Goecker

Cellular Autofluorescence Signatures for Determination of Tissue Type, Age of Evidence, and Separating Contributors from Biological Mixtures 10
Christopher Ehrhardt

Ultrahigh Speed Direct PCR. A Method for Obtaining STR Based Genotypes in Under 6 Minutes 12
Bruce McCord

(continued)

About the Editors

Nicole S. Jones, MS, is the associate director of strategic planning and operations in the Center for Forensic Sciences (CFS) at RTI International.

Erica Fornaro, AA, is a senior project management specialist in CFS at RTI International.

Contents (continued)

CONTROLLED SUBSTANCES AND TOXICOLOGY 13

Chemical Analysis of Controlled Substances Using Automated Headspace Solid Phase Microextraction—Gas Chromatography/Mass Spectrometry 14
Jorn Yu

Δ9-THC Infused Foods 15
Clark Smith

Detection and Quantification of Synthetic Opioids in Oral Fluid 16
Michael Truver and Kaitlyn Palmquist

Comparison of Two Validated LCMSMS Methods for the Quantitative Analysis of Opioids, Cocaine, and Cocaine Metabolites in Biological Matrices 18
Rebecca Wagner

FORENSIC ANTHROPOLOGY AND FORENSIC PATHOLOGY 20

Detection of Insect Stains from Four Species of Necrophagous Flies on Household Materials using Immunoassays—Development of a Quantifiable Confirmatory Test to Detect Fly Artifacts Contaminating Bloodstain Evidence 21
David B. Rivers

ICPUTRD: Image Cloud Platform for Use in Tagging and Research on Decomposition 22
Audris Mockus

Analysis of Alternative Light in the Detection of Cutaneous Bruises: A Multisite Randomized Controlled Trial 23
Katherine Scafide and Daniel Sheridan

Using Fundamental Mechanics to Predict Infant Skull Fracture Patterns 24
Brittany Coats

Abstract

The 2019 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.

Steering Committee

National Institute of Justice
Washington, DC

Gregory Dutton
Danielle McLeod
Frances Scott

Planning Committee

RTI International
Research Triangle Park, NC

Jeannine Bratts
Tchad Compton
Abby Czeskleba
Erica Fornaro
Anne Gering
Nicole S. Jones
Julian Lee
Lisa Malette
Lauren Mangum
Tyler Raible
Susan Redmond
Josh Vickers

National Institute of Justice
Washington, DC

Hannah Barcus
Gregory Dutton
Gerald LaPorte
Jonathan McGrath
Danielle McLeod
Kristin Pilgrim
Frances Scott

Project Directors

RTI International
Research Triangle Park, NC
John S. Morgan, PhD

National Institute of Justice
Washington, DC
Gerald LaPorte, MSFS

Introduction

The National Institute of Justice (NIJ) is the federal government's lead agency for forensic science research and development as well as the administration of programs that facilitates training, improves laboratory efficiency, and reduces backlogs. The mission of NIJ's Office of Investigative and Forensic Sciences (OIFS) is to improve the quality and practice of forensic science through innovative solutions that support research and development, testing and evaluation, technology, information exchange, and the development of training resources for the criminal justice community.

Through the research, development, testing, and evaluation process, we provide direct support to crime laboratories and law enforcement agencies to increase their capacity to process high-volume cases and provide needed training in new technologies. With highly qualified personnel and strong ties to the community, NIJ's OIFS plays a leadership role in directing efforts to address the needs of our nation's forensic science community.

RTI International and its academic- and community-based consortium of partnerships work to meet all tasks and objectives for the Forensic Technology Center of Excellence (FTCoE), put forward under the NIJ Cooperative Agreement No. 2016-MU-BX-K110.

The FTCoE is led by RTI International, a global research institute dedicated to improving the human condition by turning knowledge into practice. With a staff of more than 5,000 providing research and technical services to governments and businesses in more than 75 countries, RTI brings a global perspective. The FTCoE builds on RTI's expertise in forensic science, innovation, technology application, economics, DNA analytics, statistics, program evaluation, public health, and information science.

On February 19, 2019, NIJ and the FTCoE held the 2019 NIJ Forensic Science Research and Development (R&D) Symposium. This event was held in conjunction with the American Academy of Forensic Sciences' 71st Annual Scientific Meeting in Baltimore, Maryland. Hundreds of attendees joined us in person and online to learn about NIJ research awards given to several talented researchers spanning the forensic disciplines.

For more than a decade, NIJ has hosted an annual R&D symposium to showcase great scientific innovations and promote the transition of research into practice. NIJ supports research to advance efficiency, quality, reliability, and capacity in the criminal justice and forensic science communities; this research focuses on developing new technologies, providing proof for evidence-based practices, and evaluating findings for case investigations and legal proceedings.

This year, members of the NIJ R&D team—including program managers Gregory Dutton, Danielle McLeod-Henning, and Frances Scott—worked to bring you a phenomenal research agenda. The full-day program included 16 presenters and their researcher partners representing 16 NIJ awards; these awards were received during a 5-year period (2013–2017). The two morning sessions comprised Impression and Pattern Evidence/Trace Evidence and Forensic Biology/DNA; the afternoon sessions covered Controlled Substances/Toxicology and Forensic Anthropology/Forensic Pathology.

IMPRESSION AND PATTERN EVIDENCE/TRACE EVIDENCE

Quantitative Assessment of Shoeprint Accidental Patterns with Implications Regarding Similarity, Frequency, and Chance Association of Features—Empirical Chance Association of Randomly Acquired Characteristics

2013-DN-BX-K043

Jacqueline Speir
West Virginia University

The power associated with demonstrating a linkage between footwear and an impression left at the scene of a crime is directly related to the perceived rarity of the shoeprint itself, which is a function of observed class, subclass and randomly acquired characteristics (RACs). When individualizing characteristics are present, their relative position, orientation, size, and shape are examined and compared with known exemplars in an effort to establish the strength of the suspected linkage. However, the degree to which a feature might repeat by chance alone is less well understood in many pattern science fields, including forensic footwear analysis.

To inform this question, a handful of theoretical models and empirical analyses describing chance association for randomly acquired characteristics have been proposed and investigated by the forensic footwear community. Each theoretical model, although useful, is bounded by model assumptions, including independence, which have never been fully tested. Similarly, each empirical investigation has been limited by sample size, and essentially no chance associations are reported when RAC geometry is included in the comparison. However, inference suggests that with a large enough sample size, chance association must be greater than zero. In other words, RACs can and do co-occur in position, and if the geometry of the feature is simple (such as a small, isotropic, circularly shaped pinprick) then with a large enough database, an observer will find two "nondescript RACs" that coincide by chance alone. The question then becomes, "What is the magnitude of X before a chance association of 1 in X is detected?"

To empirically answer this question, the mathematical similarity of more than 3.2 million pairwise RAC comparisons was performed, as a function of more than 72,000 RACs, collected from 1,300 unrelated outsoles. The resulting similarity scores were sorted, and more than 91,000 of the mathematically most similar known nonmatch RACs with positional co-occurrence were visually assessed to determine their degree of observable similarity. Using these empirical assessments, more than 900 spatially specific chance associations are reported and available to the community via an online, open-access, and interactive heatmap at https://www.4n6chemometrics.com/database/.

Finding the Region of Origin of Bloodspatters in Complex Situations: Physical Description, New Data, Tools, and Reconstruction Method

2014-DN-BX-K036

Daniel Attinger
Iowa State University

Gunshot spatters can produce more than 40,000 stains. Here we describe the first physical models of the blood atomization related to gunshot spatters. For a backward spatter, the atomization occurs according to the Rayleigh-Taylor instability,[1] a phenomenon that also dynamically shapes volcanic eruption and supernovae. Atomization of a forward spatter is better described using percolation theory,[2] a phenomenon that abruptly fractures a dense medium like in e.g, espresso coffee making. Two open-source databases of more than 100 beating[3] and gunshot spatters are described. Impact speed, bullet shape, and distance between blood source and target wall are the main variables that are investigated. Those data sets provide the forensic community with a rich set of data for testing crime scene reconstruction models. Those data sets also illustrate the predictive capabilities of the physical models described previously. Finally, a model is described that determines the region of origin of spatters based on stain inspection, accounting for drag and gravity. The model relies on a physical description of impact dynamics and drop-flight ballistics. Implications for crime laboratories and forensic investigators are discussed.

References

1. Comiskey P, Yarin A, Kim S, Attinger D. Blood back spatter caused by a blunt bullet gunshot: theory and experiments. Bull Am Phys Soc. 2017;62. Available from: https://meetings.aps.org/Meeting/MAR17/Session/R12.4

2. Comiskey PM, Yarin AL, Attinger D. Theoretical and experimental investigation of forward spatter of blood from a gunshot. Phys Rev Fluids. 2018 Jun;3(6):063901. https://doi.org/10.1103/PhysRevFluids.3.063901

3. Attinger D, Liu Y, Bybee T, De Brabanter K. A data set of bloodstain patterns for teaching and research in bloodstain pattern analysis: Impact beating spatters. Data Brief. 2018 Mar 3;18:648-654. https://doi.org/10.1016/j.dib.2018.02.070.

Raman Microspectroscopy and Advanced Statistics for Detection and Characterization of Gunshot Residue

2016-DN-BX-0166

Igor K. Lednev
University at Albany, SUNY

Raman spectroscopy is a technique that can provide confirmatory class identification of analytes through low-intensity laser light scattering. The technique is nondestructive, rapid, sensitive, and requires little or no sample preparation. Furthermore, portable Raman spectrometers are readily available, allowing for crime scene accessibility. Raman spectroscopy offers several advantages over the current methodology for gunshot residue (GSR) analysis. The technique has been shown to detect components from both the organic and inorganic constituents of GSR on adhesive tape. This is contrary to current GSR elemental analysis methods, which rely solely on the detection of the heavy metals (lead, barium and antimony). This is problematic because environmental concerns have led to the increased popularity in heavy-metal–free or "green" ammunition. Until recently, the application of Raman spectroscopy for GSR analysis was largely unexplored, although this approach is not dependent upon detecting metals and is more capable of differentiating environmental contaminants and GSR. Therefore, a Raman spectroscopic method displays numerous advantages in specificity when compared to current techniques.

The firearm discharge process is analogous to a complex combustion reaction. Therefore, the chemical composition of the products (GSR) is directly related to the chemical nature of the reagents (ammunition) and the reaction conditions (firearm). Our preliminary results show that Raman data collected from GSR particles originating from different firearm-ammunition discharges were successfully classified. Discharge samples from 0.38 inch and 9 mm caliber firearms were probed using a 785-nm Raman excitation. Resulting data was treated with statistical methods such as Principle Component Analysis (PCA) and Support Vector Machines (SVM). Our results show a high probability that this method correctly identifies GSR from the two examined calibers. Since GSR is often collected from a suspect, the application of this method to forensic investigations would provide a link between GSR collected from the shooter and the crime scene. We have also developed a new two-fold spectroscopic mapping method for the detection and identification of GSR particles on a substrate of adhesive tape. The advantages of this novel approach relative to the current technique will be discussed.

This emerging technique illustrates the possibility for an on-scene, nondestructive, identification and chemical characterization method for GSR. This method has the potential to greatly impact the forensic science community by increasing the accuracy (and discriminatory power) of GSR detection. The most direct application for this research is a method to exclude a specific firearm-ammunition combination as producing an evidentiary GSR sample. The comparison of a laboratory-generated GSR sample discharge and an evidentiary GSR sample can be made without extensive preliminary studies.

This project was supported by Award No. 2016-DN-BX-0166 awarded by the National Institute of Justice, Office of Justice Programs, US Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.

Consistent Single Shot Detection of Organic and Inorganic Residues from One Sample using LC/MS and Host-Guest Complexes

2015-DN-BX-K048

Suzanne Bell
West Virginia University

Significant progress has been made in the past few years in developing methods for detection of organic residues (OGSR) using advanced mass spectrometry. Such instruments are increasingly affordable and available in forensic laboratories and are widely used in forensic toxicology. This presentation will describe a method that utilizes electrospray ionization/liquid chromatography/mass spectrometry to detect the elements found in gunshot residue (GSR) and OGSR from a single sample and small-volume sequential extraction. Elemental constituents were detected in the form of host-guest complexes and identified by selected ion transitions and isotopic abundances. GSR elements were consistently detected from single shots from both 0.38 revolvers and 9mm semiautomatic pistol discharges. Background and blank studies confirmed that residues were not associated with background contamination or carryover. Samples were collected using scanning electron microscopy stubs topped with a soft commercial polymer that were dabbed against the skin analogous to the collection method for scanning electron microscopy/energy dispersive X-ray spectroscopy. The polymer was sequentially extracted in a centrifuge tube first with dilute aqueous acid and then methanol. Initial studies utilized crown ethers (15-5 and 18-6) to complex cations of barium, antimony, and lead. Sample was introduced via flow injection through a C18 guard column and characterization via triple quadrupole mass spectrometry. Barium, lead, and iron were recovered from every hand swab from 1-3 shots while antimony was not recovered. Inductively coupled plasma mass spectrometry analysis confirmed that the initial digestion process was insufficiently aggressive to solubilize this element from GSR. For OGSR, the methanolic fraction was injected through a C18 column and in all cases, ethyl centralite, methylcentralite, and n-nitrosodiphenylamine was recovered. Interestingly, diphenylamine detected in only one sample, 3 shots discharged from the revolver.

Subsequent studies were undertaken to improve digestion of the antimony, explore other complexing agents, and develop a single injection mode for simultaneous detection of GSR and inorganic GSR. Current results on these studies will be presented.

FORENSIC BIOLOGY/DNA

A Method to Estimate the Age of Bloodstains Using Quantitative PCR

2014-DN-BX-K025

Robert W. Allen
Oklahoma State University

The value of RNA analysis in the forensic laboratory as one means of identifying the nature of biological evidence of forensic relevance has been well established. The degradation of RNA in dried body fluid stains has also been an area of forensic interest because of the potential to estimate the age of a stain recovered from a crime scene. Here we describe a somewhat novel quantitative polymerase chain reaction (qPCR) assay that demonstrates it is possible to estimate the age of bloodstains with reasonable accuracy. The 5'–3' qPCR assay exploits the observation the 5' end of an mRNA transcript degrades in dried stains faster than the 3' end. This differential degradation pattern can be followed with a qPCR assay that quantifies ~90 bp amplicons produced from the 5' and 3' ends of a panel of four transcripts chosen from the transcriptome of blood because of the degradation kinetics determined initially using RNA sequencing. Statistical analysis of degradation curves suggests, depending upon the age of the sample, the window of accuracy in age estimates is about 2–4 weeks for stains less than 6 months of age and 4–6 weeks for stains 6 months to 1 year old.

Proteomic Analysis of a Single Human Hair for Ancestral Classification—Use of Genetically Variant Peptides to Statistically Estimate the Genetic Background of Hair Shafts

2015-DN-BX-K065

Zachary Goecker
University of California, Davis

Recent developments in proteomic analysis offer the prospect of considerable improvement for use of hair evidence for individual identification. Individual variation in the form of genetically-variant peptides (GVPs) are a consequence of nonsynonymous single nucleotide polymorphisms that are translated as single amino acid polymorphisms. Known and well-characterized nonsynonymous mutations permit detection of GVPs in digests of hair proteins. Proteomic methods provide quantitative values with experimentally determined error rates. Currently, random match probabilities derived from protein reach into the millions for a single hair. To further maximize the usefulness of proteomic technology, two factors were explored. The first was optimization of hair processing so that a single hair fragment would suffice for a rapid analysis. We can now process less than one inch of hair in under 24 hours. The second was that processing different ancestral groups assumes that the method used is consistent across different biogeographic backgrounds.

Results testing 2 cm of hair indicate that protein digestion is improved at room temperature, reducing using 100 mM DTT, and agitating by stirring. Trypsinization for 6 hours solubilizes most of the hair by mass and results in detection of more unique peptides than with longer digestion times (>24 hr). An optimized hair-processing procedure, with shorter times for both reduction and digestion, has yielded improvements in detection of GVPs, and results in a similar number of GVPs compared to other approaches. For 2 cm of hair, powers of discrimination have reached up to 1 in 106 million, and for 4 mg of hair, 1 in 231 billion. Current work addresses proteomic equivalence between European and African protein expression levels and GVP occurrences. Because GVPs from less abundant proteins are more likely to be observed consistently with increased efficiency of hair digestion, protocol optimization is envisioned to increase the number of GVP identifications and increase resulting discrimination. Further work is also underway to automate creation of GVP profiles, and to characterize GVPs through the use of internal standards to aid in meeting the Daubert standard of evidence admissibility. This research may advance both ancestral classification and individual identification. Proteomics may bolster hair evidence as an objective identification method alongside mitochondrial haplotyping.

Cellular Autofluorescence Signatures for Determination of Tissue Type, Age of Evidence, and Separating Contributors from Biological Mixtures

2015-DN-BX-K024

Christopher Ehrhardt
Virginia Commonwealth University

Analysis of biological mixtures is a significant problem for forensic laboratories. The presence of cells from multiple individuals in a biologic stain complicates DNA profile interpretation and often leads to loss of evidence. One promising strategy is to utilize morphological and/or intrinsic fluorescence profiles (i.e., "autofluorescence") to discriminate, and ultimately separate, contributor cell populations in a mixture. Although autofluorescence signatures have demonstrated applications for clinical diagnostics, they have yet to be investigated as a tool for processing cell mixtures for DNA casework. Therefore, the goal of this study was to survey cellular autofluorescence signatures across a range of forensically relevant cell types (saliva, buccal, epidermal, blood) and deposition conditions (dried and aged between 24 hours and 2 months) and then develop a workflow for using these signatures to separate contributor cell populations from biological mixture samples. Results showed that cell populations from each of the four tissue types could be resolved with a high degree of accuracy regardless of sample age. Specifically, epidermal cells were distinguished from vaginal and buccal cells with a classification accuracy of ~94%, whereas blood cells could be differentiated from the three epithelial cell types with an accuracy over 97%.

Analysis of variable weights indicated that measurements capturing the circularity, aspect ratio, and autofluorescence between 450 and 680 nm of cells were the largest drivers of multivariate differences between tissue types. In order to demonstrate applications for DNA case working units, autofluorescence signatures were used to physically isolate contributor cell populations from both mixed tissue and single cell type mixtures prior to DNA profiling. Results from two-person mixtures consisting of buccal cells and blood cells showed near single source profiles from mixtures that had been aged for up to 72 hours and various contributor ratios (3:1, 1:1, 1:3). Similar results were obtained for single cell type mixtures of whole blood, with single source profiles obtained in mixtures with varying contributor ratios, age (up to ~96 hours), and with as many as five contributors. Another important outcome for this work is that cellular autofluorescence profiles were observed to change systematically as the sample aged and could be used to determine the time since deposition for an unknown biological sample. Specifically, the median intensity of autofluorescence detected between 350 and 680 nm increased incrementally at 24-hour time points between 0 and 7 days. Nonlinear differences were also observed between 1 week and 2 months. Autofluorescence signatures were then used to construct a multivariate framework to predict the time since deposition in an unknown biological sample. Results showed that cell populations were correctly associated with eight different time points between 24 hours and 2 months with an accuracy of ~85%.

Overall, results from this project suggest that cellular autofluorescence measurements such as these, which can be obtained in a high throughput and nondestructive fashion, can be used to separate contributor cell populations in many types of biological mixtures and simultaneously provide probative information regarding the age of an evidence sample.

Ultrahigh Speed Direct PCR. A Method for Obtaining STR Based Genotypes in Under 6 Minutes

2015-R2-CX-K038

Bruce McCord
Florida International University

Recent advances in DNA polymerases, thermal cyclers and microfluidic systems have the potential to provide the forensic DNA community with methods for rapid analysis of samples. We have been exploring the potential to increase the speed of multiplex polymerase chain reaction (PCR) through careful optimization of experimental parameters including polymerase, PCR cocktails, and amplification ramp rates. In our research experimental design methods and PCR enhancement techniques have been utilized to produce seven locus multiplex amplifications as fast as 6 minutes using extracted DNA and direct PCR amplifications (no extraction at all) in 12 minutes. The results can be visualized in under 80 seconds using a microfluidic electrophoresis system containing a heated plate with a denaturing polymer buffer. In this paper we discuss the aspects of our experimental design and the optimization of polymerase and buffer conditions. We believe that this high-speed genotyping in combination with microfluidic detection has great potential in suspect screening and other projects where rapid identification of individual suspects is necessary.

CONTROLLED SUBSTANCES AND TOXICOLOGY

Chemical Analysis of Controlled Substances Using Automated Headspace Solid Phase Microextraction—Gas Chromatography/Mass Spectrometry

2014-R2-CX-K005

Jorn Yu
Sam Houston State University

Forensic applications of automated heated headspace-solid phase microextraction coupled to a gas chromatography/mass spectrometry (HHS-SPME-GC/MS) in controlled substance analysis will be discussed in this talk. This analytical platform offers a good potential to be used as a rapid and sensitive process to capture chemical features for evidence. A variety of controlled substances in a variety of forms can be extracted with HHS-SPME. Nonvolatile analytes could be extracted well at 150° C. Incubation times ranging from 5 to 10 minutes proved sufficient for extraction and derivatization. The presence of other impurities from the headspace of evidence potentially could serve as unique identifiers for revealing formulation trends, synthesis methods, growing regions, and other information that could be of interest to forensic tasks. The heated process for SPME also demonstrated beneficial to facilitate one step headspace derivatization for less volatile analytes. Examples of using HHS-SPME-GC/MS for cannabinoids, fentanyl, synthetic cathinones, psychedelic mushrooms will be presented. We will demonstrate that the HHS-SPME-GC/MS platform for chemical analysis of controlled substances is rapid, efficient, and cost-effective. Many controlled substances could be readily extracted and detected from the headspace of evidence by HHS-SPME-GC/MS. With almost no sample preparation, the technique enables an automated process that efficiently transforms headspace chemical signature of evidence into digital data. We expect the analytical platform will be a rapid and reliable analytical testing method for the development of artificial intelligence for characterization of controlled substances.

Δ9-THC Infused Foods

2015-DN-BX-K028

Clark Smith
Denver Police Department Crime Laboratory

Delta-9 tetrahydrocannabinol (Δ9-THC) is the active chemical in marijuana that causes intoxicating effects. Colorado has an active market in which Δ9-THC is infused into foods. State regulations mandate certain manufacturing processes but clandestinely manufactured food materials remain a constant issue and the amount of Δ9-THC in food can be a criminal matter. Developing and validating a quantitative food analysis method is an important forensic tool.

City of Denver Forensics and Evidence Division has been working to develop a Δ9-THC infused foods method. Quantitation can be difficult due to varying food matrices. Research focus includes analyzing hard candies, infused liquids, and baked goods.

The presentation will focus on several techniques to aid in the analysis of Δ9-THC infused foods. Topics of research include methods and results for:

- dry ice homogenization;
- enzymatic digestion of triglycerides; and
- removing sugars and fats from the matrix, extraction solvent selection, and matrix effects on gas chromatography and liquid chromatography.

Detection and Quantification of Synthetic Opioids in Oral Fluid

2017-R2-CX-0019

Michael Truver
Kaitlyn Palmquist
Sam Houston State University

Synthetic opioids, like U-47700 and furanyl fentanyl, were introduced into the illicit drug market as heroin adulterants. As a result, the number of opioid-related deaths in the United States increased significantly. Oral fluid has been investigated as a toxicological matrix of interest as it may indicate recent drug use and may be useful for determining drug use trends. To address the limitations of routine forensic analyses, two analytical methods were developed and validated for the screening and quantification of fentanyl- and nonfentanyl–related synthetic opioids in oral fluid using liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques. Pooled oral fluid (containing 1:3 oral fluid:Quantisal buffer) was fortified with deuterated internal standards and extracted with a polymeric solid-phase extraction column. After aqueous and organic washing, analytes were eluted with 5% ammonium hydroxide in ethyl acetate and 5% ammonium hydroxide in 80:20 dichloromethane: isopropyl alcohol for the screening and quantification methods, respectively. Analytes were dried down and reconstituted in mobile phase. Comprehensive screening and quantification methods for fentanyl analogs and other synthetic opioids were developed and validated according to Scientific Working Group for Toxicology Standard Practices for Method Validation in Forensic Toxicology guidelines. The screen was performed using an Agilent Technologies 1290 Infinity liquid chromatograph coupled to an Agilent Technologies 6530 Accurate Mass Time-of-Flight mass spectrometer in two acquisition modes: TOF mode and All Ions Fragmentation mode. Personal Compound and Database Libraries were produced in house containing fentanyl analogs of interest (n = 14), as well as other drugs of abuse and additional synthetic opioids (n = 53). An Agilent 1290 Infinity liquid chromatograph system equipped with an Agilent 6470 Triple Quadrupole Mass Spectrometer was used for quantification of buprenorphine, U-47700, U-49900, U-50488, AH-7921, MT-45, W-18, W-15, and heroin markers (6-acetylmorphine, morphine). For the screening method, the limits of detection (LOD) for all fentanyl analogs in oral fluid were 0.25 ng/mL and 2.5 ng/mL in time-of-flight and all-ion-fragmentation modes, respectively. No carryover or interferences were observed. Matrix effects in oral fluid were considered acceptable for all analytes with ion suppression and enhancement ranging from -11.7-13.3%. Processed sample stability was assessed after 24 hours in the autosampler (at 4°C), and all analytes were determined to be stable, except alfentanil (>25% loss in autosampler). For the quantification method, LOD and limit of quantitation (LOQ) for all analytes were 5 ng/mL and 10 ng/mL, respectively. Linearity was determined between 10 and 500 ng/mL (R2 > 0.9959). Bias and precision were <±11.1%. Matrix effects ranged from -21.1% to 13.7%. No carryover or qualitative/quantitative interferences were detected. All analytes were stable under all conditions tested. Authentic oral fluid samples (n = 18) collected via Quantisal devices from arrestees (under a protocol approved by an institutional review board) did not contain any novel synthetic opioids. Morphine or heroin use was indicated

https://doi.org/10.3768/rtipress.2019.cp.0009.1902

in 4 cases. Additional drugs detected included methamphetamine (n = 15), amphetamine (n = 7), cocaine (n = 4), codeine (n = 1), alprazolam (n = 1), and mephedrone (n = 1). Morphine concentrations were <LOQ, 32, 104, and 146 ng/mL and 6-acetylmorphine in those same cases were <LOD, 15, <LOQ, and 110 ng/mL. These methods are currently being used to analyze authentic samples from various populations in order to help assess the prevalence of these synthetic opioids. The comprehensive screening method will also help determine drug use trends of common drugs of abuse.

Comparison of Two Validated LCMSMS Methods for the Quantitative Analysis of Opioids, Cocaine, and Cocaine Metabolites in Biological Matrices

2015-DN-BX-K008

Rebecca Wagner
Virginia Department of Forensic Science

The proliferation of misuse of both prescription and non-prescription opioids, in recent years, has caused an opioid epidemic in the United States. Forensic toxicology laboratories often encounter implications of abuse in both driving under the influence of drugs and death investigation cases. The Toxicology Section of the Virginia Department of Forensic Science (VADFS) receives driving under the influence/driving under the influence of drugs, death investigation, and other police cases for analysis. From 2012 to 2017, VADFS had a 191% increase in the number of reported opioid results and a 1439% increase in the number of reported fentanyl results for death investigation cases. The increased prevalence of drug use extends to all case types and is complicated by poly drug use. Traditionally, analyses are completed by individual drug class, which subsequently requires an individual case to be evaluated using multiple analytical techniques for comprehensive analysis. To ease the impact of ever-increasing case submissions and case complexity, VADFS has validated two liquid chromatography tandem mass spectrometry (LCMSMS) methods for the quantitative analysis of opioids, cocaine, and cocaine metabolites in biological matrices. The methods were validated in accordance with the Scientific Working Group for Forensic Toxicology method-validation guidelines and VADFS validation requirements. The methods were then compared to determine the advantages and disadvantages of each analytical technique. The newly developed methods not only require a decreased sample volume, but they also combine four analytical techniques into one method, which significantly impacts laboratory productivity. Furthermore, since implementation, the qualitative analysis of over 30 fentanyl derivatives has been validated as an addition to the opioid, cocaine, and cocaine metabolite method. Two sample preparation techniques, solid phase extraction and protein precipitation, were employed for the validation of the two quantitative positive ionization mode DynamicMRM LCMSMS methods. Aspects evaluated during the validation were accuracy and precision, sensitivity, calibration model, ionization suppression/enhancement, recovery, carryover, interferences, dilution integrity, and post-extraction stability. All compounds passed the comprehensive validation for antemortem and postmortem blood for both methods whereas urine only passed qualitative acceptance criteria. Overall, the protein precipitation indicated more ionization suppression than the solid phase extraction, but the presence of ionization suppression was determined to have minimal effect on the methods limit of detection or accuracy and precision. A comprehensive comparison of the two methods was completed using the validation data and a cost-benefit analysis. The rapid protein precipitation extraction, in conjunction with an 11-minute analysis time, significantly increased laboratory efficiency upon implementation in the Toxicology Section of VADFS. Given the prevalence of emerging fentanyl

derivatives, over 30 fentanyl derivatives have been qualitatively validated using the protein precipitation extraction to further increase laboratory efficiency. Therefore, the use of either fully validated method for the analysis of opioids, cocaine, and cocaine metabolites can aid in streamlining forensic toxicology analysis.

https://doi.org/10.3768/rtipress.2019.cp.0009.1902

FORENSIC ANTHROPOLOGY AND FORENSIC PATHOLOGY

Detection of Insect Stains from Four Species of Necrophagous Flies on Household Materials using Immunoassays—Development of a Quantifiable Confirmatory Test to Detect Fly Artifacts Contaminating Bloodstain Evidence

2016-DN-BX-0181

David B. Rivers
Loyola University Maryland

Despite claims that fly artifacts can be detected based on morphological features, alternate lighting, and presumptive chemical tests, few species have been tested by the reported methods for discernment and none have proven to be consistently reliable in distinguishing insect stains from human body stains. In an effort to overcome deficiencies in current methods used for identification of insect stains, an immunoassay has been developed that utilizes polyclonal antisera (termed anti-md3) based on a unique cathepsin D-like proteinase found in some cyclorrhaphous Diptera. The confirmatory immunoassay (dot blot) recognizes insect stains that contain fly digestive enzyme, specifically fly regurgitate and defecatory or fecal stains. In this study, artifacts produced by four species of necrophagous flies (Protophormia terraenovae, Calliphora vicina, Cynomya cadaverina, and Sarcophaga bullata) were examined using the confirmatory immunoassay to determine if insect stains could be distinguished from a range of human body fluids (e.g., blood, semen, urine, saliva, feces). Adult flies were fed ad libitum human blood, semen, urine, feces, or saliva for 24 hours at 25°C and permitted to deposit artifacts on a range of household materials: ceramic tile, carpet (plush), t-shirt (cotton), wood block, and unfinished drywall. A lift technique was developed that permitted transfer of fly artifacts from the test materials to filter paper (Whatman #4 110 mm Æ) for dot blot analyses. Artifact transfers were confirmed visually and with ALS using a 450 nm emission filter and an orange contrast filter. All species readily deposited artifacts on all test household materials regardless of diet consumed. Despite differences in texture and porosity of the household materials, artifacts of all species transferred to saturated filter paper (Dulbecco's PBS) with apparent equal efficiency based on visual inspection. With all fly species, anti-md3 sera bound to artifacts produced after feeding on semen, blood, feces, urine, and saliva. Binding appeared proportional to the size of the artifact transferred during the lifts. By contrast, none of the human fluids tested positive in the immunoassays, nor did lifts from household materials not exposed to flies. There was no evidence of false positives with any of the fly species tested, regardless of diet consumed. Similarly, there was also no indication of false negatives with any of the dot blot assays. However, flies did deposit artifacts not derived from the digestive tract on the test materials that, as expected, did not yield positive reactions with the immunoassay. Such artifacts generally cannot be visually distinguished from regurgitate and defecatory stains and thus can yield results perceived as false negatives. These observations suggest that immunoassays using anti-md3 sera coupled with a simple lift technique can be used effectively as a confirmatory assay to distinguish fly regurgitate and fecal stains from human body fluids. The new method overcomes the limitations of current techniques and can be performed reliably by anyone properly trained without the need of a forensic expert for consultation.

ICPUTRD: Image Cloud Platform for Use in Tagging and Research on Decomposition

2016-DN-BX-0179

Audris Mockus
University of Tennessee

Daily documentation of longitudinal decomposition across hundreds of human donors throughout the seasons is key to understanding the myriad of factors that can affect decomposition and hence explains the appearance of a body when it is found at a crime scene. Since 2011, the Forensic Anthropology Center has taken daily photos of all donors during decomposition at the Anthropology Research Facility, creating a database that demonstrates the effects of scavenging, insect activity, weather events and other factors that affect the pattern and rate of decomposition. The photo database now includes over 1 million images and occupies over 4 TB of disk space. Despite the promise of digitally preserving fleeting instances of decomposition, the collection is too large for manual inspection and the relevant forensic information is hidden within images, making it impossible to use for meaningful forensic and statistical analysis. An extensible online platform, called Image Cloud Platform for Use in Tagging and Research on Decomposition (ICPUTRD), has been developed to support forensic work on this digital collection. More specifically, the platform supports four of the most basic tasks a research study might undertake: explore the collection, find artifacts based on metadata, find artifacts based of forensic relevance, and curate the database to facilitate a variety of research studies. Meanwhile, the platform has to be able to handle the size of the data, be easily accessible, simple to use, and easily extensible. To satisfy these requirements, this project utilizes common web and cloud technologies such as MEAN stack (MongoDB, Express, Angular JavaScript, and Node JavaScript) and Docker containers to build ICPUTRD. After importing images and populating the database of metadata (weight, age, height, date), the next daunting task was a three-step process to extract forensically relevant information from the images themselves. By creating a nomenclature of standard terminology commonly used in the medico-legal community and applying it to tag specific parts of each photo (e.g., "eggs" or "adipocere"), the database currently contains over 1,000 images that were tagged with 5,000 terms. Furthermore, Conventional Neural Network (CNN) techniques were being implemented to train CNN over the tagged images and predict tags on the remaining images. Human decomposition experts can easily add and share standard nomenclature or study-specific tags, thus enriching the database. To determine whether the platform is achieving its goals and to find areas in which it needs to be extended, an evaluation study is underway in which 20 forensic researchers are performing searches of previously tagged images and tagging new images themselves. The preliminary results of the study are highly encouraging, with users being able to quickly find relevant forensic features. It is anticipated that ICPUTRD will become a standard tool for forensic researchers of human decomposition and will answer questions relevant to law enforcement investigations. We hope that curation capabilities that allow experts to tag additional images (and share the tags) would result in an extremely rich resource for studies of human decomposition.

Analysis of Alternative Light in the Detection of Cutaneous Bruises: A Multisite Randomized Controlled Trial

2016-DN-BX-0147

Katherine Scafide[1]
Daniel Sheridan[2]
1 George Mason University
2 Texas A&M University

Problem Statement: Victims of violence often have cutaneous bruises latent or barely visible to the naked eye due to skin color, injury age, or depth. Unidentified injuries can cause a disparity in forensic investigations, leading to unsuccessful or lack of criminal prosecutions. An alternate light source (ALS) with short narrowband visible (NBV) or long ultraviolet (UV) spectrums may improve detection and visibility of cutaneous bruises. However, the limited research available is not definitive about whether light absorption within these wavelengths results from bruising or artifact. Little is known how skin color affects bruise visibility using an ALS.

Purpose: The purpose of this study was to analyze the effectiveness of ALS in improving bruise detection and visibility over white light. We also explored the effects of skin color, gender, localized fat/muscle and bruise size, mechanism, and color on specific wavelength performance over time.

Partnership: This multisite study is a partnership between two US universities.

Subjects: Participants include 156 healthy adults, ages 18–65, with equal sampling of six skin color categories.

Methods: Using a crossover randomized controlled trial design, the order of ALS and white light application was randomized for each bruise. Bruises were created under controlled application of a paintball pellet and dropped weight to randomly selected upper and lower arm, respectively. Both bruised areas were assessed for bruise detection and visibility under white and alternate light at 21 time points over a 4-week period. A smaller subset (n = 30) of subjects continue to have their bruises assessed for up to 8 weeks. Bruise visibility using wavelength peaks within UV (365 nm) and NBV (415 nm, 450 nm, 475 nm, 495 nm, 515 nm, 535 nm) spectrums and filters (yellow, orange, red) are examined. Spectrophotometric measures of skin and bruise color are obtained along with digital photographs under each light source.

Results: Descriptive summaries of findings will be presented on this recently completed study. In addition, ALS photographic techniques developed for the study will be discussed as they will advance current practice standards.

Conclusion: ALS has the potential to improve bruise detection on diverse victims of violence and extend the timeframe for when bruises may be visible. Image analysis of bruise photographs may hold the key towards objective assessment of bruise visibility and detection under ALS.

https://doi.org/10.3768/rtipress.2019.cp.0009.1902

Using Fundamental Mechanics to Predict Infant Skull Fracture Patterns

2016-DN-BX-0160

Brittany Coats
University of Utah

Background: Approximately 686,000 children are victims of abuse and neglect every year. The highest percentage of these children are between birth and 3 years of age and are more likely to experience a recurrence of maltreatment if the abuse is not identified. Accidental falls are the leading cause of nonfatal injury in infants and the most common explanation given by caretakers suspected of abuse. Thus, distinguishing a truthful history of a fall from a false one proves to be a difficult but important task for a clinician and for the legal system. Skull fracture is a common finding for both accidental falls and abusive injuries, but it is unknown how to distinguish fracture from accidental or abusive scenarios. There is an urgent need for careful biomechanical investigations to determine characteristics of trauma that lead to specific skull fracture patterns in infants. The purpose of our research is to develop and validate a computational toolset for predicting skull fracture patterns in infants. We then plan to use the toolset to identify skull fracture patterns from common low height accidental falls in infants, and to evaluate the effect of head impact direction, impact energy, and skull thickness on skull fracture patterns.

Methods: Human infant parietal and occipital bone specimens (n = 17) were obtained from autopsy and tested in a custom three-point bending high-rate (1.66 ± 0.09 m/s) impact system to identify rate-, region-, and direction-dependent material properties. Using the resulting properties, we developed a high-fidelity computational framework to predict crack propagation following a head impact. The framework integrates three-dimensional crack growth software based on fundamental fracture mechanics to identify patterns of crack growth with finite element software for complex dynamic simulations. The framework was validated against a reported parietal skull fracture in a 5-month old infant cadaver dropped from 15 cm onto concrete.

Results: Occipital and parietal infant cranial bones were 10 times stiffer when tested parallel trabecular fibers compared to perpendicular to the fibers. Regardless of fiber orientation, parietal bone was consistently stiffer than occipital bone. Interestingly, cranial bone was only rate dependent when tested parallel to the fibers rather than perpendicular. This is similar to some fiber dominant tissues, such as tendon. The computational framework and associated workflow was successfully created, and accurately predicted the skull fracture pattern of a 5-month-old cadaver head impact to concrete.

Conclusions: Infant cranial bone is unique compared to adult cranial bone in that it has noticeable trabecular fibers that significantly influence the mechanical response of the bone. The mechanical effect of these structures is 7 times greater than originally believed. These data led to the development of a successful computational framework for predicting infant skull fracture patterns. Future studies with the framework will parametrically evaluate the effect of impact energy and impact direction on skull fracture patterns in children and provide the medical and legal communities with empirical data to improve medical and judicial accuracy in child abuse cases.

RTI International is an independent, nonprofit research institute dedicated to improving the human condition. We combine scientific rigor and technical expertise in social and laboratory sciences, engineering, and international development to deliver solutions to the critical needs of clients worldwide.

www.rti.org/rtipress **RTI Press publication CP-0009-1902**