PROCEEDINGS

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Contents

Northeastern Association of Forensic Scientists

Drug Chemistry Abstracts

"*DART-MS Collision Induced Dissociation (CID) for Structural Analysis of Synthetic Cannabinoids*" Rabi A. Musah[1], Marek A. Domin[2], Robert B. Cody[3],Ashton D. Lesiak[1], A. JohnDane[3], and Jason R.E. Shepard[*1] [1]Department of Chemistry, University at Albany, State University of New York (SUNY), 1400 Washington Ave., Albany, NY, U.S.A. 12222. [2]Mass Spectrometry Center, Merkert Chemistry Center, Boston College, 2609 Beacon Street, Chestnut Hill, MA U.S.A. 02467-3808 [3]JEOL USA, Inc., 11 Dearborn Rd, Peabody, MA 01960, U.S.A.

The emergence of numerous cannabinoid designer drugs has been tied to large spikes in emergency room visits, overdoses, and fatalities. Identifying these substances is difficult due to (1) the fact that the compounds are novel, structurally related, and do not usually test positive in drug screens; (2) the rapidity with which they appear on the market; (3) the absence of standard protocols for their identification; and (4) the customized and extensive sample preparation/extraction and analysis procedures required to demonstrate their presence. Direct analysis in real time mass spectrometry (DART-MS) is a technique that utilizes an atmospheric pressure ion source that produces a heated stream of metastable helium species directed at sample surfaces to vaporize and ionize liquids or desorb and ionize molecules from solid surfaces in open air under ambient conditions. Semi-volatile substances, like the cannabinoids mixed on the plant material, desorb from the leaf surface and are ionized. The metastable helium atoms initiate a cascade of ion-molecule reactions in ambient air to provide protonated water clusters as a chemical ionization reagent to produce parent ions, $[M+H]^+$, from the cannabinoid drugs.

DART-MS provided high mass accuracy, to 0.0001 Da, establishing the presence of the cannabinoids JWH-122, JWH-203, JWH-210, RCS-4, and AM-2201, alone and as mixtures of at least two cannabinoids. Mass spectra were acquired by simply suspending a small portion of sample between the ion source and the mass spectrometer. The ability to test minute amounts of sample is a major advantage when limited amounts of evidentiary material are available. This method circumvents time-consuming sample extraction, derivatization, chromatographic and other sample preparative steps required for analysis by more traditional methods. The high throughput capabilities of DART-MS enable the active components of designer drugs to be detected more quickly, reducing the time necessary to triage analytical evidence. Therefore, exploitation of this method has the potential to contribute to more timely criminal prosecution. In addition, DART-MS employing CID conditions provided confirmatory structural information that was useful in characterizing the various isobaric cannabinoid analogs.

Specifically, DART-MS CID induces fragmentation of the protonated parent ions when the electrode voltage at the inlet orifice to the mass spectrometer is increased, demonstrating the utility of fragmentation patterns for distinguishing among closely related structures. Our group used in-source CID to produce product ions corresponding to the synthetic cannabinoid molecules desorbed from dried leaves. CID analysis illustrated that closely related compounds are likely to fragment in a similar

fashion, but their inherent structural differences will result in unique fragments that vary enough between the singular cannabinoids that can serve as a means to better identify each substance. Closely related compounds fragmented with both consensus peaks and unique fragments, such that both their structural similarities and differences provided multiple diagnostic peaks that permitted additional confidence towards identification of each substance. DART-MS spectra were acquired to rapidly differentiate among synthetic cannabinoids contained within "herbal" products purchased locally in New York (U.S.A.).

"*Advanced Handheld Narcotics Screening*" Michael D. Hargreaves, Lin Zhang, Wayne Jalenak, Robert L. Green, Craig Gardner, Thermo Scientific Portable Analytical Instruments, Thermo Fisher Scientific

Advances in handheld instrumentation are providing significant additional capabilities for unknown chemical identification in the field. Rather than removing a sample from the hazard zone, whether from a specific area/location or container, responders may now rely on handheld instruments for rapid identification directly in the hot-zone, saving time and increasing responder safety.

The rapid, non-destructive identification of chemicals, including items of interest to the military and law enforcement by, rugged, portable Raman and FTIR spectroscopy is discussed. The techniques have been widely deployed and are now used frequently by the military, homeland security personnel, police and forensic personnel, mostly due to the high chemical specificity which allows robust identification of bulk contraband materials.

The presentation will discuss the application of the latest generation handheld Raman device, for the application of narcotics identification/screening in field. Several examples will be discussed highlighting the power of Raman spectroscopy, specifically Thermo Scientific TruNarc for the identification of street narcotic samples.

Keywords: Raman, Handheld, identification, narcotics

"*Synthetics 2012*" James F. Wesley, Monroe County Crime Laboratory, Rochester, NY

As the list of available synthetic cathinones and cannabinoids keeps expanding, crime labs are forced to spend large sums of money to acquire these compounds in order to identify the substances. First we recap the synthetics which have been introduced to the world market in 2011 and 2012. We then discuss issues regarding identification, specifically regarding E.I. mass spectra and retention time, and problems with cathinone isomers. The possible utilization of other reasonably affordable methodologies to enhance the identification scheme is then discussed. Issues of probable cause regarding law enforcement street identification with current test kits will also be covered. Our excel spreadsheet containing all currently available synthetic compound spectra (sorted by key ions) will be provided as a first step in the identification process. Attendees are encouraged to bring spectra of new or novel substances to the talk for inclusion in a shared database to be distributed to all.

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"*Analysis of Synthetic Cannabinoids, Cathinones and Meth Labs by Solid Phase GC-IR*" Tom Kearney, Spectra Analysis Instruments Inc

The number of isomers and analogs of synthetic cannabinoids and synthetic cathinones has increased dramatically as "manufacturers" try to avoid existing laws by making slight modifications to the parent structure. These molecular modifications make the unequivocal identification of these substances difficult as the isomers are often indistinguishable on GC-MS. Solid phase GC-IR is an excellent analytical technique for spectral identification of these compounds. Solid phase GC-IR spectra of closely related isomers and analogs will be presented.

"*2012 SWGDRUG Update*" Jack Mario, Suffolk County Crime Laboratory/SWGDRUG Committee Member

The presentation is a review of the activities of the Core Committee of SWGDRUG for two meetings in 2012. Topics reviewed include three published documents SD-3, involving the uncertainty determination of balance weighings, SD-4, uncertainty of quantitative (drug purity) determinations, and SD-5, reporting formats. There will be mention of other issues discussed including training for seized drug analysts, the ENFSI sampling calculator, reference materials and chemical analogues.

"*Synthetic Designer Drugs: Is it an Analog? How do you know?*" Lindsay E. Reinhold, M.F.S., F-ABC, NMS Labs, ACECSA Chair, Heather L. Harris, M.F.S., J.D., D-ABC, Functional Group Forensics, Terry R. Stouch, PhD, Science for Solutions

This workshop is designed to introduce the forensic chemist to the current issues surrounding the synthetic designer drugs including the scientific approach to analog determination being developed by the Advisory Committee for the Evaluation of Controlled Substance Analogs (ACECSA). At the conclusion of this session, attendees will understand relevant scientific concepts proposed by the ACECSA to comprehensively evaluate non-controlled substances as potential analogs of controlled substances and assist in the scientific prosecution or defense of analog drug cases.

The scientific method developed by the ACECSA will benefit the forensic science community by providing laboratories with objective, science-based criteria to evaluate compounds and a means to establish consistency in analog determinations made in laboratories across the country.

The Advisory Committee for the Evaluation of Controlled Substance Analogs (ACECSA) was established by scientists from federal, state and private forensic laboratories, academia and law to develop a scientifically valid and peer-reviewed means of evaluating the analog status of non-controlled substances and serve as a resource to law enforcement, legal counsel, laboratories and government agencies in the scientific categorization of non-controlled substances. The Committee was gathered intentionally to maintain an independent, un-biased, and un-weighted stance in the scientific and legal communities. The main goal of constructing this group was to address a lack within the forensic chemistry field regarding the evaluation of analogs. To date, there are no guidelines, recommendations or methods that exist in our field and no consensus or consistency in the determination of these compounds. Scientifically-

sound guidelines or recommendations for analog determination are needed in the forensic arena in response to the overwhelming "designer drug" explosion and the difficult task of legislating potentially harmful new drugs.

The members of the ACECSA, in collaboration with national and international subject-matter experts, developed five aspects of a compound that should be included in evaluating analog status: Chemical Structure, Physicochemical Properties, QSAR/Computational Chemistry, Synthetic Pathway, and Toxicology/Pharmacology. The panel experts will present applicable concepts and associated acceptance criteria to demonstrate the comprehensive approach to analog determination.

"Non-Chromatographic, Direct Sampling Analysis: A Novel Approach to the Mass Detection of a Wide Range of Analytes in Various Matrices with Little or No Sample Prep" **Carl Schwarz, Avinash Dalmia, & Hayley Crowe; PerkinElmer**

A novel, enclosed Direct Sample Analysis (DSA) ion source using field free atmospheric pressure chemical ionization (APCI) coupled to a time of flight (TOF) instrument was developed for the direct and rapid analysis solids, liquids, and gases. DSA-TOF uses no chromatography so results occur in seconds. This technique produces rapid high resolution, high mass accuracy data in seconds with little or no sample prep. DSA-TOF can be potentially implemented as a screening or confirmation tool in the forensic lab environment. The device can be rapidly removed and replaced with a liquid chromatograph to perform LCMS experiments where retention time and high resolution quantitation are required.

Forensic crime labs are responsible for the qualitative confirmation of drugs of abuse for use in court cases. Gas chromatography mass spectrometry (GCMS) has been the gold standard for confirmation illicit drugs. GCMS analysis generally requires extraction, derivatization, and long run times. DSA-TOF potentially eliminates these steps. Here, we present a workflow that produces superior results in seconds in which the resulting data could arguably be more defendable in a court of law. Subsequent to some non-mass spectrometry screening process of drug collected from seizures, DSA-TOF provides a rapid confirmation. Both targeted, for the confirmation, and non-targeted drugs can be identified in seconds with little or no sample prep. In addition, TOF produces spectra and thus results that can always be reviewed at a later time for other analytes of interest.

We will also present examples of arson constituents and gunshot residues. Lastly, DSA-TOF was employed to identify potential markers for the adulteration of juices. A similar workflow could be used in other forensic applications.

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Collegiate Competition Abstracts

"*Forensic Investigation of PSU Herbal Incense Street Samples using GC-MS and LC-MS/MS*" Sharyn E. Miller, Julie McIntosh, and Dr. Dan Sykes, The Pennsylvania State University

Legal herbal products, found readily available through the Internet and local gift shops, are increasingly being used for recreational drug use by youth. Marketed as herbal incense and specifically labeled "not for human consumption," these products are plant materials sprayed with cannabinoid-related chemicals that users vaporize and inhale.[1,2] Two commercial herbal samples, Wet and Down2Earth Climax, purchased in downtown State College, PA, are investigated and analyzed to determine synthetic cannabinoid presence and quantity. JWH-018, JWH-073, JWH-200, CP-47, 497, and cannabicyclohexanol are analyzed, as these represent five(5) chemicals currently placed on Schedule I classification by the US Drug Enforcement Administration (DEA).[2] Scheduling was in response to an increase in the frequency of hospitalizations involving incense inhalation in the United States.[3] In addition to the five Schedule I chemicals listed above, 30 other synthetic cannabinoid related chemicals are being investigated in order to construct a library for instrumental drug screening.

To accomplish synthetic cannabinoid chemical screening, multiple extraction techniques were compared; the QuEChERS (Quick, Easy, Cheap, Rugged, and Safe) extraction method[4] proved to be most suitable. QuEChERS provides a time effective option by combining the herbal sample with magnesium sulfate and calcium chloride buffering salts and methanol solvent in a 50mL centrifuge tube. After shaking the sample for 5 minutes, sample centrifuging ultimately allows for removal of the organic phase. Extracts are characterized using a Shimadzu QP-2010 GC-MS and an Applied Biosystems 3200 Triple Quadrupole LC-MS/MS. Optimized methods, coupled with library construction of synthetic cannabinoid standards, enable simultaneous screening of cannabinoid species, and permit a comparison of the two instrumental setups for drug screening and quantification of street herbal products.

Currently, employee drug testing does not incorporate these compounds, but as more analogs become illegal and available, screening will be forced to expand to include such chemicals. Thus, it is important to develop and validate instrumental methodology for such screening. Traditional crime laboratory drug analyses focus on GC-MS instrumentation, but semi-volatile and thermally unstable compounds may not be suitable. LC-MS/MS methods may prove more suitable for such chemicals. Preliminary results indicate a rapid, effective method for the separation and identification of synthetic cannabinoid standards and commercial herbal samples for GC-MS and LC-MS/MS. Using the method of internal calibration, deuterated analogs are utilized to quantify the synthetic cannabinoids in the samples. Products from the

same brand allow for determination of inner-batch variability. Future analyses will expand to incorporate other street samples from the area, and to evaluate sample heterogeneity. Pending method optimization, the research project can be expanded to include other emerging cannabinoid-based drugs that come into vogue.

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"Novel analysis and characterization of gunshot residue via Raman spectroscopy combined with advanced statistics"

Justin Bueno, Vitali Sikirzhytski, Igor K. Lednev, University at Albany, SUNY

A new method for gunshot residue (GSR) analysis, identification and discrimination is being developed. The technique implements Raman microspectroscopy, an advantageous form of evidentiary analysis due to its non-destructive nature, and rapid and portable capabilities. The method is augmented with the addition of advanced statistics to differentiate experimental Raman spectra collected from non-equivalent GSR samples. This research has the potential to greatly impact the accuracy and effectiveness of shooting incident investigations, by providing a potential chemical and statistical link between GSR collected from the shooter and the crime scene.

Preliminary results illustrate Raman's ability to detect both organic and inorganic components of resulting GSR.[1] These components were linked to substances found in the original ammunition, prior to the firearm discharge. This is contrary to current GSR analysis methods which rely solely on the detection of the heavy metals (lead, barium and antimony). This dependence is problematic since environmental concerns have led to the increased popularity in heavy metal free or "green" ammunition. It has been found that in the absence of heavy metals, current GSR analysis techniques are severely hindered when making accurate identification of GSR samples. It is our hypothesis that the firearm discharge process is analogous to a complex chemical reaction. Varying the reagents (ammunition) of such a reaction affects the chemical and morphological nature of resulting products (GSR particles). By treating Raman data with advanced statistics, we have successfully achieved a proof of concept for this idea. Raman data collected from GSR particles originating from different firearm-ammunition discharges were successfully classified according to caliber size. 0.38 inch and 9 mm caliber firearm discharge

samples 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 of the method to correctly classify the data from the two examined calibers.

This emerging technique illustrates the possibility for an on-scene, non-destructive, identification and chemical characterization method for GSR. The most direct application for this research is a method to exclude a specific firearm-ammunition combination as producing an evidentiary GSR sample. The comparison of a laboratory generated GSR sample discharge and an evidentiary GSR sample, can be made without extensive preliminary studies.

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"*The Analytical Investigation of Synthetic Street Drugs*" Amanda Leffler; Frank L. Dorman; Phil Smith; Adriana De Armas, The Pennsylvania State University

Stores across the country are carrying new products sold as bath salts, plant food, and jewelry cleaner. These products contain designer drugs, which have become prevalent in the United States since 2009 when they came from Europe.[1] The drug names (i.e. bath salts) illustrate the variety of marketing tactics that manufacturers employ to lure customers. Products exhibit colorful packaging labels and every bag states the product is "Not for Human Consumption" to allow for legal possession and consumption by circumventing potential control mechanisms.[2] Not only are these substances sold in head shops, but they are carried in gas stations, adult stores, independently owned convenience stores, and online retailers.[3] The internet has provided a significant means of circulating new compounds quickly and effectively. In March 2009, there were reportedly fewer than ten online vendors; by June that number had grown to dozens, with new sites opening every week.[4]

Synthetic cathinones are entering the drug market faster than they can be restricted. Analysis of such designer drugs and the identification of individual compounds may help ban their production and abuse. By the time enough information is known about a drug to place it under temporary or permanent scheduling, replacement compounds have already been created and readied for distribution. A quick and efficient extraction method will allow for a more rapid analysis of such compounds. Identification of these drugs can help increase knowledge on their pharmacology to provide better treatment options and possibly decrease the number of calls to poison control centers across the country. In regards to the forensic community, crime labs already have a large workload. An efficient methodology will benefit analysts by increasing laboratory throughput.

This presentation will discuss the development of the extraction of the drug compounds from various commercial media, followed by separation using gas chromatography with mass spectrometric detection (GC-MS) and liquid chromatography with time-of-flight mass spectrometry (LC-TOFMS). The developed chromatographic method provides qualitative and quantitative analysis of synthetic compounds in street samples based on the use of appropriate standards. A preparatory HPLC method for the fractionation of multi-component samples and the use of direct infusion MS/MS in further identification of unknown samples will also be discussed.

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"*Statistical Comparison of Black Ballpoint Pen Inks Using Hyperspectral Imaging and Principal Component Analysis: An Update*" Kaitlin Hafer, Cedar Crest College; Daniel Zamzow and David Baldwin, Ames Laboratory

Hyperspectral imaging (HSI), a nondestructive technique, provides spectral and spatial data of a sample of interest. One potential application is to differentiate ink types with similar physical properties by collecting images of the ink on paper at a range of different reflected wavelengths. This technique may prove useful in cases where questioned document examination is required. HSI may be useful to determine whether alterations have been made to documents such as letters, checks, passports and drivers licenses using a different ink than used by the original writer. When images collected using a range of different wavelength interference filters to filter detected reflected light are compiled together, an image dataset is created in which the behavior of the sample, with respect to each wavelength, can be visualized. In this study, statistical differentiability was determined for image datasets produced by HSI with a noncommercial imaging system. Ten bandpass filters ranging from 550nm to 1000nm were used in combination with a Leica Universal Forensic Microscope (UFM-4) equipped with a Hamamatsu CCD camera to create datasets for 41 black ballpoint pen inks written individually on filter paper. The hyperspectral data of the black ballpoint inks were analyzed using principal component analysis (PCA) to perform pair-wise comparisons and determine which pixels were distinguishable within a 95% confidence interval (CI). Plots of the fraction of pixels within this confidence interval for the pair-wise comparisons

can be used to determine a threshold for matching. Alternatively, receiver operating characteristic (ROC) plots were generated to compare and optimize the algorithms used in this work.

"*Evaluation of Direct PCR Amplification*" Daniel Hall, Dr. Reena Roy, The Pennsylvania State University

Current methods often involve PCR amplification directly from body fluid that is bound to substrates with the requirement that the sample-stained substrate first be washed several times with purification reagents and extraction buffer. The wash and dry procedure requires a minimum of one to three hours depending on whether the process is manual or automated. The process is time consuming and risks the possibility of sample contamination.

The objective of this study is to deposit blood and saliva samples on different types of substrates. Once the samples are dry 1.2mm punches will be obtained from the samples described above and amplified using direct amplification procedure without subjecting them to washing and extraction steps. In this study types of substrate, amount of reagents needed, and if necessary amplification parameters (thermal conditions) will be varied to detect autosomal and Y-STR DNA profiles using the following nine different amplification kits: AmpF STR® Identifiler® Direct, AmpF STR® Identifiler® Plus, AmpF STR® Identifiler®, AmpF STR® YFiler®, PowerPlex ® 16 System, PowerPlex ® 16 HS System, PowerPlex ® 18D System, PowerPlex ® 21 System, and PowerPlex ® Y23 System.

The major issues that will be addressed in this research are the following:
1. Do all eight of the substrates yield similar robust DNA profiles?
2. Do all four kits yield similar robust DNA profiles?
3. Do all primers and sample types require the recommended reaction volume?
4. Do all saliva and blood samples yield similar robust DNA profiles?
5. Can the thermal cycling conditions be changed to yield better results from substrates that do not appear initially satisfactory?

"*Chemical Analysis and Characterization of Synthetic Cannabinoids in Herbal Incense Products*" Ashton D. Lesiak and Rabi A. Musah, University at Albany, SUNY, Albany, NY; Marek A. Domin, Boston College; Robert B. Cody and A. John Dane, JEOL USA, Inc; Jason R.E. Shepard, University at Albany, SUNY

The emergence of cannabinoid designer drug use has been tied to an increase in visits to hospitals and emergency rooms. Patients have reported anxiety, tachycardia, and myocardial infarction upon ingesting the substances and synthetic cannabinoids have been implicated in several cases involving fatalities. These substances are often difficult to identify because the compounds are new, closely related in

structure, and as such are not included in conventional drug screens. Moreover, new cannabinoids rapidly appear on the market, making it difficult for officials to stay ahead in restricting the entire range of substances.

Six locally obtained samples of "herbal incense" (Spice, K2) were analyzed for synthetic cannabinoid content. Samples were preliminarily screened by Direct Analysis in Real Time mass spectrometry (DART-MS). DART-AccuTOF-MS provides high mass accuracy, enabling formula weight determination down to 0.0001 Da. DART-MS allows for rapid analysis of these synthetic cannabinoids, tested directly under ambient conditions on the plant material, with no extraction, derivitization, or sample preparation whatsoever. The six samples were run against standards and determined to contain the following cannabinoids alone or in combination: AM2201, JWH-210, JWH-122, RCS-4, and JWH-203, or their close isomers/analogues. In addition, a Soxhlet extraction was performed to solubilize the synthetic drugs from the plant material. The results from the DART-MS analysis were confirmed by gas chromatograph-mass spectrometry (GC-MS), compared against standards, and matched to the SWGDRUG library. Samples containing AM2201 and JWH-210 were quantitated and the Spice samples varied in concentration from 26 mg/g to 141 mg/g of AM2201 and 4 mg/g to 38 mg/g of JWH-210. DART-MS provided rapid analysis of the cannabinoids found in the herbal incense in a manner that is not possible with traditional analytical processes.

"*Evaluation of Athletic Supplements Containing Dimethylamylamine by Direct Analysis in Real Time-Mass Spectrometry*" Kendra J. Adams and Colin Henck, University at Albany, SUNY; Marek A. Domin, Boston College; Robert B. Cody and A. John Dane, JEOL USA, Inc; and Jason R.E. Shepard, University at Albany, SUNY

Athletic supplements have been widely used among professional athletes, military personnel and recreational weight lifters throughout their existence. Many compounds found in these supplements have been banned due to their dangerous nature or the unfair advantage they give the user. Dimethylamylamine (DMAA), a compound that has been reported to be found naturally in the geranium plant, is one such drug that has been proven to be extremely detrimental to users. It has caused false positives for amphetamine drug tests, and has been associated with instances involving cerebral hemorrhages and even fatalities. Due to emerging information about health risks of this drug, dimethylamylamine has been under high scrutiny by several governments, including Australia and New Zealand, where it has been identified as a party pill. These countries have already placed a national ban on dimethylamylamine and it can no longer be sold, purchased or used in athletic supplements. In May of 2012, the United States Food and Drug Administration sent a letter to all supplement companies using dimethylamylamine in their products requiring them to provide appropriate safety information regarding this compound.

Several athletic supplements containing dimethylamylamine were purchased at popular retail stores such as GNC and Vitamin World as well as through various online supplement retailers. Supplements used for analysis were, Jack3d, OxyElite Pro, Hydroxystim, Ripped Juice EX2, DNPX, Dexaprine, and Hyde Pre-workout Amplifier, among others. Samples were analyzed via two methods. Analyses were performed directly on the solid powders with Direct Analysis in Real Time mass spectrometry (DART-MS). In addition, solid formulations were solubilized and extracted to obtain free base of DMAA from the salt form of the compound included in each product. The solutions were also analyzed by gas chromatography-mass spectrometry. However, DART-MS analysis of the solid form of the supplements directly identified the active ingredients and many other additives, providing unique spectra for each formulation and avoiding extensive sample preparation, derivitization, or extractions. DMAA was confirmed to be present in all of the samples tested to date. Testing will continue on other nutritional supplement products to determine DMAA content.

"*Identification of the "Legal High" Phenylalkylamine Analogues: 5-Iodo-2-Aminoindane (5-IAI) and 5, 6-Methylenedioxy-2-Aminoindane (MDAI) by Colorimetric Tests and GC-MS*" James S. Joseph and Adam B. Hall, Boston University School of Medicine

Over the past decade, the illicit drug market has experienced an explosion of designer drugs being produced by clandestine laboratories that include delineations from traditional illicit drugs that dominated drug markets for long periods of time (e.g. cathinones and MDMA). These designer drugs, which are commonly known as "legal highs", are popular due to that fact that they are legally obtainable and not currently controlled. Examples include the phenylalkylamine analogues 5-IAI and MDAI, which have similar biological effects to MDMA. In spite of the unknown risk factors associated with these substances, "legal highs" continue to have high levels of interest amongst recreational users. As such, the potential for abuse is high, and 5-IAI and MDAI are under consideration in numerous jurisdictions for regulation.

Many of these novel compounds have never been analyzed previously within a forensic setting. The chemical and physically properties of 5-IAI and MDAI are not fully understood. As a result, the analytical analysis of "legal highs" can be challenging. Color test kits provide a quick screening method for law enforcement officials looking to presumptively identify a substance within the field. The difficulty with this form of analysis is that most of the active ingredients present in "legal highs" are not detected by standard, presumptive tests or their results are ambiguous. Gas chromatography-mass spectrometry (GC-MS) is one of the most utilized analytical instruments in forensic laboratories for the identification of drugs of abuse. However, due to the rapid development and commercialization of "legal highs," the limited availability of certified reference standards and mass spectral data make the confirmatory analysis of "legal highs" challenging.

The primary aims of this research were two-fold. The first was to evaluate selected commercially available Narcotics Analysis Reagent Kits (NARK® II) and color reagent formulations recommended by the National Institute of Justice "Color Tests Reagents/ Kits for Preliminary Identification of Drugs of Abuse" to determine if the phenylalkylamine analogues 5-IAI and MDAI generate a color development. If a color was generated using a particular reagent, further testing was conducted to establish if the observed color would be detectable in the presence of various adulterants. The second aim of this research was to develop a rapid GC-MS method for the detection of 5-IAI and MDAI in contrived multi-component mixtures of selected adulterants.

Standard color tests provided consistent results for 5-IAI and MDAI pure samples as well as mixtures with adulterants. 5-IAI produced a light brown color with Marquis and MDPV color reagents. Mandelin reagent produced a greenish brown color with the NARK® II individual test kit and a light green color with the in-house preparations of the Mandelin reagent when tested with MDAI. Confirmatory analysis was performed using GC-MS with a temperature gradient. The analysis was performed on a non- polar (5% phenyl) methylpolysiloxane column with a total run time of 10 minutes. 5-IAI and MDAI were chromatographically separated and distinguishable from various adulterants based on retention time and mass to charge ratio.

"*Weeding Analytes Out of Marijuana: The Identification and Quantification of Pesticides in Cannabis Utilizing Comprehensive Gas Chromatography*" Emily Ly, Jack Cochran, Julie Kowalski, and Frank L. Dorman, The Pennsylvania State University

This presentation will impact the forensic science community as well as humanity in a variety of different ways, but loosely falls into two classes: characterization of Cannabis as a potential pharmaceutical, and potentially fingerprinting the trace compounds in Cannabis to determine the point of origin. Testing for potency using gas chromatography with flame ionization detection (GC-FID) can help determine the identity and abundance of target cannabinoids (cannabinol, cannabidiol, cannabichromene, cannbigerol) that have therapeutic qualities. These qualities have been confirmed to relieve pain, control nausea, stimulate appetite, and decrease ocular pressure[5]. With this knowledge, medical marijuana can be grown more effectively by lessening the main psychoactive component THC, which may cause discomfort in patients[7], and increasing the target therapeutic cannabinoids. The abundance of THC and other cannabinoids are affected by a variety of factors including environmental conditions, harvesting periods, and the sex of the plant[1]. Furthermore, many pesticides, fungicides, and insecticides are used to treat the cannabis plant. This is of concern for any person that consumes the material due to the residual toxins that are potentially harmful. Moreover, a study was conducted in 1992 for the United States Drug Enforcement Administration (DEA) where it was determined that chemical profiles of Cannabis samples could be used locate the geographical origin[4]. However the system could only eliminate possible sources of origin and therefore had low specificity due to the fact they only analyzed cannabinoid constituents[4]. By identifying and quantifying the pesticides on the Cannabis plant, it may be possible to develop a

"chemical fingerprint" relating to compounds used by growers to increase the crop yield. This information may allow law enforcement agencies determine and/or link the source location of the confiscated illicit drug.

"*Identification of Insertion/Deletion Polymorphisms from Evidence Samples*" Rebecca Klein, Dr. Reena Roy, The Pennsylvania State University

Analysis of short tandem repeats (STRs) is currently the most commonly used method for human identification. However, DNA extracted from evidence samples exposed to environmental insults does not always yield complete STR profiles. Light, humidity, elevated temperatures, and bacterial or fungal contaminants all degrade DNA, which can lead to the loss of genetic information. Also, the efficiency of the PCR amplification process is reduced when inhibitors such as salts, heme in blood, indigo dye found in denim, phenolic compounds, melanin from skin and hair, humic acid from soil, and collagen and calcium in bone are present in the extracted DNA. Degradation and inhibition can lead to loss of signal, peak imbalance, and allelic dropout with current STR technology. In situations where DNA is highly degraded, the molecule becomes fragmented and the chances of obtaining complete profiles are reduced. Typically, the larger amplicons are the first to fall below the detection limit. This problem has prompted research in the area of extraction and amplification methods to obtain complete DNA profiles from these compromised samples.

The current study focuses on the detection of insertion/deletion polymorphisms from challenged samples using the Investigator DIPplex® kit from Qiagen. Unlike the PCR amplification kits currently available in the forensic community that amplify 15 or more STR loci, the DIPplex® kit allows for multiplex amplification of 30 bi-allelic areas of known insertions and deletions (InDels) plus the Amelogenin locus. These unlinked markers are distributed across 19 chromosomes and the PCR products are no larger than 150 base pairs.

This PCR amplification kit uses reduced amplicon sizes similar to SNPs, improving the amplification of degraded samples. Due to the small amplicon size, the assay is highly sensitive and the manufacturer claims it can create a full DNA profile from as little as 63 pg of DNA. The combination of current STR analysis procedures and small amplicon sizes makes InDels suitable for pristine as well as degraded DNA evidence samples.

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"*Frequency of Somatic Mutations in the STR Region of Human DNA amongst Three Sample Types and Their Effect on Genotyping*" Lizzie Martin, Cedar Crest College

The frequency and effect of somatic mutations of Short Tandem Repeats (STRs) used in forensic typing are not known and their existence in loci used in forensic genotyping could have significant implications. If somatic mutations exist in these loci, sources of DNA from various matrices could potentially yield different genotypes. As a consequence, the type of matrix (for instance, saliva, blood, and hair) must be considered when determining the appropriate exemplar for comparison with evidentiary samples. In order to evaluate the impact of somatic mutations on forensic genotyping, three biological matrices of DNA from each of approximately 75 Caucasian participants were genotyped. Blood, hair, and saliva samples were subjected to organic extraction followed by quantitation with the Sigma-Aldrich® SYBR® Green JumpStart™ Taq ReadyMix™ kit using a Qiagen Rotor-Gene 6000 real-time PCR instrument. The samples were then amplified and genotyped using the Life Technologies™ AmpFLSTR® kit on a Life Technologies™ 3500xL Genetic Analyzer. The profiles generated were analyzed with Life Technologies™ GeneMapper® ID-X software. Within the small sample size of less than 100 participants analyzed to date, there has been no occurrence of somatic mutations. The occurrence of these mutations is more likely to be found within a larger population size, as in general more mutations are likely to be found when more samples are analyzed. In the future, evidence of somatic mutations will be verified through sequencing to negate the possibility of the existence of mutations in primer binding sites used during the amplification process. A frequency determination will then be used to evaluate the findings.

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Criminalistics Abstracts

"*Geographic correlation of drug-use patterns with currency drug contamination*" Chun-yu Chen, Frederick P. Smith; Forensic Science Department, University of New Haven, West Haven, Connecticut

Known drug-use prevalence from widely distributed geographic and demographic locations were evaluated for correlation with analyses of currency bills for the presence of methamphetamine, amphetamine, methylene dioxymethamphetamine (MDMA), ketamine, morphine, and cannabinoids.These results were compared with other, international reports on currency contamination, with ananalysis of their forensic implications, specifically furthering the understanding of external contamination issues, which may be germane to medico-legal investigators who evaluate the significance of drug test results in (1) questioned deaths, (2) public health concerns/forensic epidemiology, (3) drug crimes, (4) drug-use toxicological tests where reliability is based on assumptions about external contamination, particularly when forensic investigators rely on examinations of surfaces in the workplace and elsewhere to interpret the significance of the presence of drugs on these surfaces. Convenience stores, hotels, fuel stations, entertainment and gaming locations served to represent diverse geographical locations and commercial enterprises, from which currency bills were collected. A minimum of 10 bills were collected per location from 20 different locations. Proper specimen collection/custody was observed in order to ensure that test results reflected pre-collection contamination, rather than sample handling by researchers.

Immunological test strips capable of detecting 50 ng cut-off concentrations of the following classes of controlled substances/metabolites: cannabis, amphetamines, cocaine, and opiates were used to perform presumptive testing. Extraction with 0.1 N HCl was used for instrumental confirmation. Laboratory contamination was examined along with positive and negative controls for method validation and to test that positive test results did not arise from laboratory conditions. Quantification relied on the addition of 100 ng each of the deuterated internal standards (d3- cocaine, d5-amphetamine, d5-methamphetamine, d5-methylenedioxymethamphetamine, d4-ketamine, d3-morphine, and d3-Δ9-tetrahydro- cannabinol) to specimens.

Analytes were extracted from currency with10 mL 0.1 N HCl followed by solid phase extraction (SPE) and derivatization with 50 uL 0.1 triethylamine in methylene chloride, 50 uL pentafluoroproprionic anhydride at 70oC for 30 min. Excess derivatization reagents were evaporated under a stream of nitrogen. Drugs were reconstituted in 20 uL of ethyl acetate and 2 uL aliquots were injected into the ion-trap GC/MS. When extracted and screened, a significant number of bills showed positive results for cannabis (69.6), amphetamines (65.2), opiates (26.1), and cocaine (8.7). The relationship between illicit drug seizures and drug-use demographics were compared to evaluate the probative value to forensic epidemiological purposes. Results from currency bills show quantities of controlled substances (ng/bill) which exceed the limits of detection and limits of quantification for analyses of other external surface matrices used in workplace drug testing and other forensic settings, for samples such as hair (expressed

in ng/mg) and sweat (expressed in ng/mL extract), as well as inanimate objects, for which there is no mandated standard practice or cutoff for reporting a surface as "positive" for a drug. These results suggest that more research is needed to provide the scientific underpinning necessary to ascribe drug use or drug possession conclusions in a forensic setting based on surface testing, particularly when contamination cannot be excluded as the source.

***"An Approach to Discrimination and Match Quality of Questioned and Known Fiber Comparisons and using Databases to Provide a Statistical Significance Estimate of Fiber Associations"*; Edward G. Bartick*, Ph.D, Kevin Roberts; Forensic Science Program, Department of Chemistry and Biochemistry, Suffolk University, Boston, MA; Stephen L. Morgan, Ph. D., Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC; John . Goodpaster, Ph.D., Forensic and Investigative Sciences Program, Indiana University, Purdue University, Indianapolis, IN**

The statistical variance measurements of like and unlike fibers is critical to how the estimations of match significance can be made from fiber evidence. Multivariate statistical analysis, databases and the application of the product rule of independent variables was used to establish the significance of matches.

The ability to classify and discriminate cotton and synthetic fibers is described. A library of over 800 well characterized fibers was developed with known dye components. Using polarized light microscopy, U /visible microspectrophotometry (MSP) and Fourier transform infrared spectroscopy (FTIR), data was collected on fibers. In this report, studies were done on twenty-one red cotton and twenty-one red acrylic fibers using multivariate analysis. The absorption spectra of fibers from 10 replicate U /visible microspectrophotometry scans on each fiber were compared by using principal component (PCA) and linear discriminant analysis (LDA). The software used in this work was developed at the University of South Carolina. PCA is used to find the directions of maximum variability to reduce the data dimensionality and enable use of LDA to provide projection maps of the data providing best discrimination of fiber groups. After projection into a two- or three-dimensional discriminant map, discrimination of fiber groups can be judged visually by drawing 95 confidence limit ellipses around each group of points representing replicate spectra from the same fiber. Additional statistical hypothesis testing with Hotelling s T2 test for the equality of means can be employed as a match criterion. With the aid of multivariate statistics, fibers that are difficult to distinguish by visual comparison can be distinguished. PCA and LDA were also used to compare like fibers to determine how well fiber color matched on similarly dyed fibers. The gold standard in forensics is the approach used in DNA matching by calculating the probability of occurrence of a given combination of alleles in short tandem repeats by the product rule of probability. By knowing the number of fibers in the database with specific color, diameter, cross-sectional shape, and chemical composition, the percentage occurrence of each fiber was determined. The product of the percentages was then calculated to determine the probability of two fibers matching randomly with those characteristics. Probabilities on the order of 1 in 0.5 million are obtainable with such comparisons between fibers, provided a sufficiently large and representative database of fiber characteristics is accessible. The improved understanding of sources of variability and decision-making processes gained from this research will serve to advance the forensic significance of class evidence involving fiber examinations.

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"*Misinterpretation of the Science by Just Being Human*" ; Susan Ballou; National Institute of Standards and Technology, Gaithersberg, MD

Foundational research of forensic science disciplines is needed to bolster current practices conducted throughout the forensic science profession. The National Institute of Standards and Technology (NIST) Law Enforcement Standards Office (OLES) has initiated research projects toward advancing the underlying measurement science of several topic areas. The key areas that have been subjected to extensive research at NIST are ballistics, latent prints, & arson.

At NIST the Ballistics Imaging Database Evaluation (BIDE) project was initiated to determine the feasibility & integrity of a national ballistics imaging database. The initial focus soon diverged into several research avenues; one avenue identified human variables that caused poor image capture while another avenue demonstrated the inadequacies of software programs when un-compressing stored images.

The Expert Working Group on Human Factors in Latent Print Analysis initiated by OLES was put into action after a series of high profile cases highlighted the fact that errors can occur, albeit at a very low rate. The point of the working group was to develop an understanding of the role of human factors & their contributions to errors in latent print analysis. This was followed by a determination of a means to reducing errors in terms of cost, feasibility, associated risks, & the quality of the evidence.

Fire pattern repeatability research at NIST will contribute to the fundamental understanding of burn patterns & thereby aid fire investigators at all levels. The modeling of the source fires & the resulting burn patterns is demonstrating the need for new training in fire origin identification.

This presentation will highlight each of the above topics demonstrating the aggregate research results to the practitioner and management. The revealing research results are just the start to remedy the weak link; which is being human, and to show education & recognition of these issues are essential.

"*Environmental Forensic Investigation to Identify Contamination Sources of Polycyclic Aromatic Hydrocarbons in a Stream With Automated Sample Preparation Techniques*"; Melinda T. Pham, Frank Dorman, Cedric Neumann, and Jack Cochran; Pennsylvania State University, 107 Whitmore Laboratory, University Park, PA; Jessica Netzer; J2 Scientific, 1901 Pennsylvania Drive, Suite C, Columbia, MO

Modern society has increased the usage and varieties of organic compounds for various benefits; however there can be a negative side of the use of these same chemicals – environmental exposure. The "contaminant" may be transported great distances through the ecosystem before detection. In addition, once detected, the compounds may undergo significant degradation, metabolism, or other processes termed weathering. This may make the identification of the original source of the pollution more difficult. It is the identification, quantification, and determination of the source or sources of

environmental pollution that largely comprise the science of environmental forensics, and the subject of this presentation. The organic contaminants of interest in this study are persistent organic pollutants (POPs) like polycyclic aromatic hydrocarbons (PAHs).

PAHs are organic, atmospheric pollutants created through the incomplete combustion of fossil fuels like coal and petroleum. These airborne pollutants can travel a great distance before depositing onto surfaces via wet and dry deposition. For this presentation, PAHs deposited into and around a nationally recognized fishing stream, Spring Creek, are investigated. PAHs are of environmental concern because of their persistent, bioaccumulative and carcinogenic properties, thus, identifying and monitoring the contributions of PAH sources becomes important.

The PAH emission sources have distinct signatures, but the complex environmental matrix interferes with the analysis. To eliminate interfering compounds in the sediment samples prior to analysis, the automated sample clean-up procedure will be presented. The cleaned samples are then analyzed via gas chromatography/mass spectrometry (GC/MS) for identification and quantification.

Besides matrix complexity, sediment samples contain a collection of PAHs from natural and anthropological sources. Thus, differentiating between the two will be beneficial for environmental forensics application. Field sediment samples from Spring Creek are analyzed to identify PAH sources and their apportionment. The PAH emission sources and their contributions are identified using a receptor model called Positive Matrix Factorization (PMF). PMF is a multivariate statistical tool used to identify contamination sources and the contribution of each source in a given sample. PMF was chosen for this investigation because of the ability to assign experimental uncertainties to individual data points. Secondly, solutions are constrained to non-negative values, thus, overcoming limitations found in other, current receptor models like Principle Components Analysis (PCA). The work specifically discussed in this presentation may be applied to numerous other forensically relevant samples, and this will also be discussed.

"*Visualizing Latent Fingerprints Using Columnar Thin Films*"; Stephanie F. Williams, BS, and Robert Shaler, PhD; Pennsylvania State University, Forensic Science Program, 107 Whitmore Laboratory, University Park, PA; Drew P. Pulsifer, MS, and Akhlesh Lakhtakia, PhD, DSc; Pennsylvania State University, Department of Engineering Science and Mechanics, 212 EES Building, University Park, PA

By the conformal-evaporated-film-by-rotation (CEFR) technique, a columnar thin film (CTF) is deposited onto a latent fingerprint on a substrate that is affixed to a rotating platform in a vacuum chamber. In the vacuum chamber, a source material is evaporated by resistive heating to produce a collimated vapor flux. The vapor condenses on the rapidly rotating substrate, thereby creating a thin film that entombs the fingerprint. This thin film comprises columns with diameters on the nanometer length scale. The CTF produces an observable contrast between the fingerprint ridge detail and the underlying substrate, and thus allows the fingerprint to be visualized by its surface topology rather than by mechanical or chemical interactions.

Several different studies are being conducted to test the abilities of this new technique to develop latent fingerprints. A study was carried out to determine the sensitivity of the CEFR technique by using

depletion series. For each series, sebaceous secretions were collected on a pre-cleaned finger and then fingerprints were deposited without renewal of the sebaceous material on the finger. A study was performed to test the ability of the CEFR technique to develop the latent and the bloody portions of partially bloody fingerprints. Sebaceous secretions were collected on a pre-cleansed finger and one drop was blood placed on the finger and then the fingerprints were deposited. Each substrate for each study was prepared for split fingerprints, with one half of the fingerprint being developed by the CEFR technique and the other half by a traditional technique. Substrates used in the studies included white nylon, black nylon, black abs, white abs, brass, stainless steel, white grocery bag, clear sandwich bag, and black garbage bag. Traditional methods used include powders, magnetic powders, fluorescent powders, cyanoblue with cyanoacrylate fuming, cyanoacrylate fuming, and amido black. Evaporant materials used for CTFs include gold, nickel, chalcogenide glass, tris(8-hydroquinolinato)aluminum [Alq3], and (1,10-Phenanthroline)tris[4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedionato]europium(III) [Eu(TTA)3phen].

Results obtained were qualitatively and quantitatively examined. Qualitatively, photographs of the developed fingerprints are visually graded, and quantitatively the photographs were examined using the Universal Latent Workstation (ULW). The fingerprints examined with the ULW were graded using a custom algorithm which calculates the amount of definitive minutiae in each fingerprint as determined by the quality map produced with the ULW s extended feature set.

Results obtained from the depletion study showed that brass was qualitatively superior to that of the traditional method, stainless steel was qualitatively and quantitatively superior to the traditional method, and hard plastics have mixed results. The partially bloody fingerprint study results show that the CEFR have the ability to develop both the bloody part and the latent part of the fingerprint and are qualitatively superior to traditional methods.

Further research is still being conducted in all these studies. Using the CEFR technique to develop latent fingerprints on fired cartridge casings will be a future study. The CEFR technique will also be compared with vacuum metal deposition using the split print method in the future.

This work was supported by Grant No. 2010-DN-B -K232 from the U.S. Department of Justice.

"*Comparison Study of Commercial Electrostatic Lifter and Student Made Lifter*"; Julie McIntosh; The Pennsylvania State University, Department of Chemistry, 330 Whitmore Laboratory, University Park, PA; Sharyn E. Miller; The Pennsylvania State University, Department of Forensic Science, 330 Whitmore Laboratory, University Park, PA; Kenton Chodara; The Pennsylvania State University, Department of Forensic Science, 330 Whitmore Laboratory, University Park, PA; Clare Hubbard; The Pennsylvania State University, Department of Forensic Science, 330 Whitmore Laboratory, University Park, PA; Dr. Dan Sykes; The Pennsylvania State University, Department of Chemistry, 330 Whitmore Laboratory, University Park, PA

This study investigates and compares two electrostatic lifting devices, a commercially-available product (Dustprint Lifter made by Lightning Power Company) versus one built by Penn State (PSU) students.

Electrostatic lifting is a technique employed by crime scene investigators as a method for collecting dust footprints off of multiple surfaces. A qualitative experiment was conducted to compare the commercial lifter and the PSU lifter side by side on three surfaces: tile, hardwood floor, and carpet. The quality of prints lifted, quantity of prints obtained, and ease of use between the two instruments were observed. The experimental method involved cleaning the surface, covering a Nike running shoe with corn starch, and walking fifteen total steps. Previous research investigated the maximum distance (number of steps) that can be detected from the initial position of the person to be eleven steps. This study indentified and lifted print (step) numbers 9, 11, 13, and 15 between both devices. Each lifted print was photographed using a Nikon D40 camera. Specific points on the shoe were used as individualization markers in order to compare prints lifted from both devices. For each surface, three trials were completed to determine the variability between runs.
Examination of the prints from the tiled surface indicate that both lifters collect a high-quality print at 13 steps but the quality diminishes rapidly following this step. Further analyses will explore the remaining two surfaces. The largest difference between the two devices was the ease of use and safety. The commercial lifter is significantly more difficult to use than the PSU lifter, requires the use of an outlet, and poses a greater safety risk to the user by electric shock. The PSU electrostatic lifter provides comparable qualitative results to that of commercial devices currently in-use today.

"*Raman Spectroscopic Analysis of Bone*"; Gregory McLaughlin and Igor K. Lednev; Department of Chemistry, University at Albany, 1400 Washington Avenue, Albany, NY

Recently, two publications demonstrated the applicability of Raman spectroscopy to specific problems in forensic anthropology1,2. These articles target the most central questions of the field, species and age determination of a bone in question. The positive results indicate that vibrational spectroscopy can be incredibly informative in this context. In the first article, a bone was monitored in a controlled burial environment with a Raman spectrometer and measured in short duration windows. The buried bone was measured between 2-10 weeks at roughly two week intervals. The spectra obtained from the cortical area of the bone segments shows the dramatic changes in the tissue, even from short burial durations. Furthermore, spectroscopic trends appeared that were time dependent. A preliminary model was constructed using peak integration of Raman bands, which shows a high correlation to the burial duration. The mechanism which causes this trend is unknown, but microbial action is explored as a possibility.

In the second experiment, Raman spectroscopic data was used to discriminate bone samples originating from four different species (bovine, porcine, turkey and chicken). A PLS-DA model was constructed which completely discriminated these classes. The model was constructed using the signatures of the main constituents of bone, collagen and bioapatite. This indicates that there may be species specific bone compositions, which can be effectively probed by Raman spectroscopy.

These results demonstrate the capabilities of Raman spectroscopy toward analyzing bone tissue and the potential for this technique to complement existing methodology.

1 McLaughlin, G. & Lednev, I. Spectroscopic Discrimination of Bone Samples from arious Species. *American Journal of Analytical Chemistry* **3**, 161-167 (2012).

2 McLaughlin, G. & Lednev, I. Potential application of Raman spectroscopy for determining burial duration of skeletal remains. *Analytical and Bioanalytical Chemistry* **401**, 2511-2518, doi:10.1007/s00216-011-5338-z (2011).

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DNA/Biology Abstracts

"*An Expanded Multiplex for New Global Standards*" Danielle J. Brownell, Ann MacPhetridge, and Benjamin Krenke, Promega Corporation

As DNA databases continue to grow and international cooperation increases, the need for a common set of markers is required to facilitate data sharing and to reduce adventitious matches. Promega's PowerPlex® Fusion System provides all of the materials needed for co-amplification and five-color fluorescent detection of 24 loci (23 STR loci and Amelogenin), including the CODIS core loci and the European Standard Set (ESS) loci. The PowerPlex® Fusion System will enable increased discriminatory power and data sharing possibilities by means of the incorporation of common and informative loci used throughout the world. In addition, the PowerPlex® Fusion System builds upon recent advances in Promega STR chemistries, including improved inhibitor resistance, faster cycling time, and direct amplification from a variety of common sample types, resulting in more meaningful analyses for both casework and databasing efforts.

"*New automated sample processing and analysis setup solutions implementation strategies and data from database and casework forensic DNA laboratories*" Lois Tack[1], Bas de Jong[2], Yogesh Prasad[3], Jeff Hahn[4], and Laurent Baron[5]

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Forensic laboratories are often faced with a wide variety of sample types for database and especially casework processing. Samples are collected using a diverse array of simple cotton swabs, paper swipes, FTA cards and several commercial collections devices. Typically, labs will initially test different forensic DNA vendor kit methods and/or in house chemistries manually to assess DNA quantity and quality for their range of sample types and collector kits. Based on initial testing results, one procedure set may be chosen to validate one sample subset and a different process chosen for another subset. It is common for labs to utilize four different lysis conditions for their different sample types – and then utilize different STR profiling chemistries for each of these sample subsets. Thus, a major automation challenge today is to provide different user-friendly, reliable and robust solutions to handle the many sample preparation and analysis methods in use at various crime labs.

To meet this challenge, Hamilton has developed several forensic robotic workstations that provide specific, and often one or more solutions for a given casework and/or database crime lab. We will describe implementation strategies and show validation data for various new sample preparation and analysis robotic forensic DNA systems in use at several US and global database and casework laboratories. Experimental setup and results will be presented for each of the following solutions:

(1) Combined FTA card puncher and liquid handling system with integrated high resolution imaging and barcode scanning on a single hands-free platform; the easyPunch STARlet® is ideal for direct amplification database labs and in use at the French National Police in Lyon France.

(2) Unique single platform lysis system that fully automates sample lysis using a proprietary filter spin tube in 24-tube rack format (up to 96 samples per batch) with full ID traceability. The AutoLys STAR® generates cleared lysates processed in a single and in use at the Netherlands Forensic Institute.

(3) New single DNA extraction and PCR setup platform in partnership with Life Technologies HID Professional Services to automate the forensic DNA process using tested ABI kit methods. The ID STARlet® automates PrepFiler™, Quantifiler® Duo, normalization importing 7500 data sheets, and Identifiler® Plus & Yfiler STR setup methods for 3500XL and 3130 Genetic Analyzer profiling.

(4) Kit neutral DNA extraction and PCR setup workstation using common DNA kits such as Promega, QIAGEN, ABI, and others. Data from the Forensic STARlet® is in use at Sorenson Forensics and the Kansas Bureau of Investigation (Promega Powerplex® 18D) will be shown.

(5) Small-size, low cost dedicated 12-plate PCR or CE setup system with enclosure. The NIMBUS is in use at Sorenson Forensics and San Bernardino County Sheriff crime lab.

These automated robotic workstations were introduced by Hamilton Robotics in 2011 and early 2012. Now, we have data from several crime labs demonstrating how each provides a targeted solution to increase DNA sample traceability, DNA quality and throughput.

"*Interesting DNA Case Presentation*" Alicia E. Bondi, New York State Police Forensic Investigation Center, Albany, NY

On the night of March 19, 2011, 19-year-old Dutchess County Community College student Kathryn Filiberti was attending a party with friends in Hyde Park, NY. At around 3am, she left the party alone to walk home along route 9G. At noon the following day, her body was found partially submerged in a creek in Greentree Park. At the scene and autopsy, the coroner determined that Kathryn had been sexually assaulted and strangled.

Evidence that was submitted to the NYSP Forensic Investigation Center for serology testing included a sexual offense evidence collection kit with intimate swabs and fingernail scrapings from Filiberti, and her clothing and jewelry. Serological testing found many items to be "sperm-positive," including her vaginal swabs, her boot, her jeans and her jacket. STR DNA testing revealed a "John Doe" profile that was found throughout many of the clothing cuttings, as well as in her intimate swabs, fingernail scrapings and the swabs of her face and chin. Without any other physical evidence, eyewitnesses or informants, the investigators and District Attorney's office were relying mainly on this DNA evidence to solve the murder.

Over the next 5 months, the Dutchess County Sheriff's Office submitted over 40 DNA control samples. These samples came from Filiberti's roommates, party-goers, the group of boys who found her body, and various other suspects from the area. A batch of controls was submitted August 4, 2011 which included a buccal swab from a local newspaper deliveryman, Stephen Shand. On August 9, the data revealed that the DNA profile from 23-year-old Shand was a match to the "John Doe" profile in the case. Shand was arrested and indicted by a Grand Jury on August 11.

Through additional STR and Y-STR DNA testing, further connections between Shand and the victim were uncovered. On May 6, 2012, Stephen Shand pled guilty to murder in the first degree and predatory sexual assault. At sentencing on June 21, Shand received 40 years to life in prison, a combined sentence of 25 years to life for murder and 15 years to life for sexual assault. Lead prosecutor Edward McLoughlin (Assistant District Attorney, Dutchess County) expressed his gratitude both in the media and directly to the analysts involved for all the hard work in this case, noting that it was a "nearly unsolvable" crime which was solved through DNA evidence.

"*A Simple Method for Addressing ISO 17025 Trend Analysis*" Jay A. Caponera, New York State Police Forensic Investigation Center, Albany, NY

Maintaining laboratory accreditation is crucial to the continued success of forensic laboratories whether public or private, but addressing the myriad audit standards can be a challenge. Section 5.9 of ISO 17025 requires that a laboratory have quality control measures in place for monitoring the validity of test procedures, and that data be recorded in such a way that trends are detectable. Such monitoring for trend analysis also needs to be planned and reviewed so that incorrect results are not reported. Accordingly, the Bioscience section of the NYSP Forensic Investigation Center has implemented trend analysis methodology for detecting data trends in both DNA quantitation and CE. This work provides solutions for meeting the ISO standard for forensic DNA labs with minimal time and effort.

"*Detecting shadow peaks in forensic DNA analysis*" Jay A. Caponera and Darrin Pellerin, New York State Police Forensic Investigation Center, Albany, NY

Incomplete denaturation of amplification products either prior to electrokinetic injection or during sample migration may result in artifacts that make casework interpretation difficult. This co-migration of dsDNA and ssDNA STR peaks (shadow peaks) during CE is a well-established phenomenon, and is typically caused by over-amplification, poor quality formamide, or lack of heat denaturation. While addressing these root causes can substantially reduce the occurrence and severity of shadow peak formation, such artifacts may still be observed. To aid forensic DNA analysts in identifying shadow peaks, we have developed an Excel-based calculator that predicts dsDNA migration based on linear regression with the ssDNA parent peak location. This work demonstrates that the migration of any given shadow peak is tightly linked with the putative parent peak, and that shadow peak detection may be easily predicted.

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"*Y-STR: Casework Examples and Interpretation Strategy*" Allyson D. Goble and Jay A. Caponera, New York State Police Forensic Investigation Center, Albany, NY

The Biological Science section of the New York State Police Forensic Investigation Center has been performing Y-STR analysis in casework for approximately one year. In this time, we have obtained probative results in multiple case types, including sexual assaults, homicides, and property crimes, and have had the opportunity to present our results in court. Along the way, we have encountered mutations, microvariants, and complex mixtures, and our interpretation strategy had continued to evolve. Our results indicate that usable Y-STR profiles can be obtained from a variety of sample types and that traditional serology screening may not predict these results. We have also found that mixtures should be interpreted cautiously and that a reliable male quantitation system is imperative for these analyses.

"*Validation Studies to Better Understand and Predict the Behavior of Y-STR Casework Samples*" Lynn Schneeweis, Kristen Sullivan, Guy Vallaro, Sharon Walsh, and Marisa Westlin, Massachusetts State Police Forensic and Technology Center, Maynard, MA

In 2003, Y-STR analysis was validated for casework use in the MA State Police Crime Lab DNA Unit using Reliagene's Y-Plex 6 kit. During this time, forensic Y-STR analysis was in its relative infancy and due to limitations of the technology, only a small number of MA State Police cases were analyzed using Y-STR analysis. However, our laboratory recognized the potential of this technology for many cases where there was no alternative method available to obtain useable results. Over the last 9 years, significant advances have been made in the area of Y-STR analysis throughout the forensic community that have had direct impact on our laboratory's interpretation and use of Y-STR technology. In 2007, we approved Applied Biosystems AmpFlSTR Yfiler™ kit for use on the 3130xl instrument and since then have had great success on many casework samples. As more complex cases have been processed using this technology at our laboratory, it was recognized by the MSP DNA Unit that supplementary Y-STR validation studies would be beneficial to assist in the interpretation of Y-STR results. Specifically, we have performed additional experiments that further address the areas of Y-STR mixtures including the reliable designation of major/minor contributors, the predicted behavior of each locus relative to one another at varying target amounts, and the effect of significant amounts of female DNA on the Y-STR amplification of both single source and mixture samples. This presentation will highlight our current work in this area and discuss how we hope to incorporate the results into our existing interpretation guidelines, with the ultimate goal of providing more consistency and confidence among analysts in the interpretation of Y-STR results at our laboratory

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"*Making sense of Y-STR mixtures: A Tool for Querying Y-STR Mixtures Using the U.S. Y-STR Reference Database*" Stefany Harman, Christine Lemire, Lynn Schneeweis, Kristen Sullivan, Guy Vallaro, and Sharon Walsh, Massachusetts State Police Forensic and Technology Center, Maynard, MA

In 2010, SWGDAM issued Interpretation Guidelines for Autosomal STR Typing, which included a directive indicating that a laboratory "must perform statistical analysis in support of any inclusion that is determined to be relevant in the context of a case." This directive reinforced a previous requirement by the courts in the Commonwealth of MA that for any DNA matches/inclusions to be admissible in trial (regardless of whether autosomal or lineage markers were used) they must be given a weight/significance. While imperative for providing the trier of fact with a basic understanding of the significance of a match/inclusion, this requirement provides challenges for cases involving Y-STR mixtures with no clear major/minor distinction. When a sample is comprised of a single haplotype, it is relatively simple to estimate a weight/significance of a match. This is accomplished by querying the questioned haplotype against a reference database and, using the counting method, reporting the number of observances in an appropriate population group and applying a confidence interval to correct for database size and variation. For mixture samples, however, all haplotype combinations that may comprise a mixture must be considered and accounted for when providing a weight to an inclusion. Given the large number of combinations that may be possible in even a relatively uncomplicated mixture, this task is likely to be too cumbersome to accomplish without the aid of computer software. In MA, Y-STR analysis is routinely performed on casework samples, often resulting in indistinguishable mixtures. In order to address the need for a weight or significance in such cases, we have designed a tool using Microsoft Excel that allows all possible haplotypes that comprise a Y-STR mixture to be identified and queried against the U.S. Y-STR Reference Database. This presentation will provide a brief overview of Y-STR testing in MA, the decision to bring such a tool online, outline the specific criteria we considered when designing this tool, and discuss our success with the use of the tool so far and considerations for future improvement.

"*The M-Vac System DNA Collection Device* " Jared Bradley, M-VAC Systems, Inc.

Crime, and criminal behavior, is constantly changing, and society must work hard to stay ahead. Advances in DNA testing technology have been exponential, but the backlog of unsolved cases continues to expand. One challenge is the ability to collect sufficient DNA material from the evidence, especially when the evidence is on a porous or rough surface. The M-Vac addresses this challenge by aggressively applying wet-vacuum forces to collect the target DNA material. Compared to the traditional sampling methods, such as swabbing, the M-Vac System collects significantly more DNA material from porous surfaces and scenarios where the DNA material is spread over a large area.

The M-Vac System has been around for a while, but is a relatively new sampling method in forensics. The system is designed to be an alternative or enhancement method, especially when the traditional methods have failed to produce a viable profile, rather than a replacement for the swab or cutting methods. As a new and more sensitive DNA collection method, the gap in capability is what the M-Vac System addresses, particularly in tough cases such as touch DNA or a stain on a coarse surface like concrete or brick. These surfaces are examples of substrates that are exceptionally difficult to collect

forensic DNA from. Blood stains, saliva and other biological fluids may go untested or undetected when on rough and porous surfaces because either the surface tears up the swab - rendering it useless - or the swab is unable to get into the cracks and crevices where the majority of the DNA material resides. The M-Vac System, with its spray-vacuum combination, will enable the user to reach those difficult areas and collect the target DNA material.

Sorenson Forensics in Salt Lake City, Utah, was the first to validate this process. The validation showed that in most cases the M-Vac recovered considerably more DNA than either cutting or dual swabbing. For example, saliva on polyester fabric yielded 149.2ng of DNA using the M-Vac versus 3.6ng from swabbing. Since the initial validation, Sorenson has used the system in several cases where traditional methods had not produced a DNA profile, but the M-Vac obtained enough material for at least a partial profile. In one example, after swabbing failed, the M-Vac was used on a piece of clothing from a homicide and possible sexual assault victim. Despite the victim being submerged between 8-10 hrs, the M-Vac collected sufficient DNA to generate a partial profile. Clearly, in difficult cases such as this touch DNA case, the M-Vac is a critical tool in maximizing DNA results.

"*The 21st Century Laboratory: The Challenge of Going Paperless*" Tim Stacy, STaCS DNA

The goal of this presentation is to examine the benefits and challenges of attaining the paperless DNA laboratory.

This presentation will impact the forensic science community by presenting the progress DNA labs in the US have made in their efforts to reduce their dependence on paper, the process a laboratory can use to determine the highest value processes to migrate to paperless and the role of software systems in saving a lab money while maintaining or even improving performance levels on their path to paperless.
With the advent of computers, scanners and increasingly shrinking storage devices, we have felt the need to eliminate paper from our lives. Where it is virtually impossible to eliminate paper completely, it is reasonable to plan to reduce paper from the process. Laboratories can successfully balance paper and paperless. A laboratory should endeavor to understand where it is the most appropriate, efficient and cost-effective to migrate to paperless. The laboratory must seek to understand where the value is for them and not predicate their decisions on what others have done or what they perceive to be a paperless-path.

This presentation will examine the levels and degrees of being paperless of several laboratories. DNA laboratories have a wide range of goals with regards to becoming paperless. Some have developed their own software or spreadsheets while others have implemented a commercial off-the-shelf (COTS) Laboratory Information Management Systems (LIMS) and DNA processing software, which they may have customized to meet their requirements. These solutions are designed to create and store informatics effectively; however it is still up to the laboratory to decide how efficient and cost-effective they want to be. Labs often look at the initial cost of a software solution and balk at the price without analyzing the total cost. Metrics must be captured to understand the investment in current processes so that the potential savings resulting from migrating to a LIMS can be quantified. The removal of steps which provide questionable value can lead to significant savings in salary-hour alone. A case study will be presented describing the use of DNA processing software and its effect on DNA laboratories in achieving a desired level of paperless.

It is possible for a DNA lab to achieve its desired degree of paperless and many labs are experiencing success. Instrument maintenance, quality control testing, and sample processing are all tracked using software. This eliminates the need for paper log forms. As samples move through the lab, the processes and scenarios are tracked and recorded. An audit trail is associated with each sample. These audit trails can be assembled into reports. The information is maintained in the software and can be recalled at any time. DNA labs can have a complete 360° solution that eliminates the need for printing.

"*Development of a "Global" STR Multiplex for Human Identification Analysis*" Jeff Sailus, Nicola Oldroyd, Dennis Wang, Julio Mulero, Siddhita Gopinath, Matthew Ludeman,Wilma Norona, Lisa Calandro, and Lori Hennessy, Life Technologies

National DNA databases are one of the most efficient and effective tools to provide intelligence about unknown perpetrators in criminal investigations. Due to their overwhelming success in solving crimes, governments around the world have implemented an ongoing expansion of DNA databases; for example, the European community expanded their set of standard loci in 2008 and the CODIS Core Loci Working Group published recommendations to expand the CODIS core loci set in the United States in 2011. In addition to DNA database expansion, countries are attempting to establish a legal basis for exchanging DNA database profiles between countries in criminal investigations.

Life Technologies is responding to these initiatives by developing a new STR multiplex that incorporates as many of the loci utilized in different DNA databases as possible into a single amplification reaction. This "Global" STR multiplex is larger and more discriminating. It can reduce the likelihood of adventitious matches, increase international data compatibility and improve discrimination power to assist missing person cases. The "Global" STR multiplex concept features two kits, one optimized for casework samples and the other for single-source applications while sharing the same configuration. The new chemistries will enable unprecedented capabilities in terms of efficiency, robustness, concordance and overall ability to recover information from forensic samples. Some key features are expanded allelic ladders to assist genotyping of rare alleles, inclusion of the DYS391 marker to provide gender confirmation in Amelogenin Y-deficient males, and the addition of extra primers to reduce rare instances of false homozygosity.

For Forensic or Paternity Use only.
Keywords: STR, GlobalFiler, DNA Database

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Toxicology Abstracts

"*Discovery-Based Analyses of Wastewater Samples for Characterization of Drug Usage*" Adrienne Brockman*, Masters Candidate-Forensic Chemistry, Pennsylvania State University, Eberly College of Science, 107 Whitmore, State College, PA 16802; Jack W Cochran, Restek, 110 Benner Circle, Bellefonte, PA 16823; Michelle Misselwitz, Restek, 110 Benner Circle, Bellefonte, PA 16823; and Frank L. Dorman, Ph.D., Pennsylvania State University, Eberly College of Science, 107 Whitmore, State College, PA 16802

The information obtained through the monitoring of waste water treatment facilities (WWTFs) may be used for a variety of purposes. The objective of this research is to determine compounds present in wastewater samples specifically obtained from the Pennsylvania State University wastewater treatment plant, which is being used as a control facility to refine analytical methodology. Rather than beginning with a target compound approach, a discovery analysis approach was chosen to try and determine as many compounds as possible prior to any compound list restriction. The difficulty in this approach can be the resulting complexity of the analysis. For this reason both Comprehensive Gas Chromatography coupled with Time-Of-Flight Mass Spectrometry (GC x GC-TOFMS) analysis and also High Performance Liquid Chromatography coupled with Time-Of-Flight Mass Spectrometry (HPLC-TOFMS) analysis were chosen for their inherent ability to characterize these potentially complex samples more successfully compared to other possible techniques. Once the discovered compounds are identified and quantified, the ultimate goal is to determine when and where these compounds were introduced into the wastewater system. Many compounds such as biological components, household chemicals, and drugs are expected to be found in the wastewater, as this water contains anything flushed down toilets and sent down drains. Although a large array of compounds could be identified in the wastewater, this research project will focus on drugs and drug metabolites that may be present.

Samples of wastewater were gathered by "grab" sampling from the Penn State WWTF. Multiple four-liter samples were gathered from each of the following: the influent flow, effluent flow, three intermediate stages, and final spray effluent. Following USEPA method 3510c, a liquid-liquid extraction process was performed to demonstrate a "baseline" to compare other extraction methods to. A separatory funnel was used for extraction purposes, with methylene chloride as the solvent. Immediately following, the Kuderna-Danish technique was used to concentrate the samples. Once the samples have undergone the clean-up process, analytical systems will be used to identify and quantify the present compounds.

"*An Investigation of the Binding of Benzodiazepines to Human Serum Albumin and the Effect on Quantitation in Blood Samples*" Kristina McNerney*[1] B.S., Marianne Staretz1 Ph.D [1]Forensic Science Program, Department of Chemistry and Physical Sciences Cedar Crest College, 100 College Dr, Allentown, PA 18104

Benzodiazepines are central nervous system depressants which are commonly prescribed in many different medications including sedatives, hypnotics, amnesiatics and anticonvulsants. Due to the large amount of prescriptions for benzodiazepines being issued in the United States they are increasingly being used as recreational drugs, often in combination with other drugs such as opiates and alcohol. It is also very commonly being seen in drug facilitated sexual assaults due to the ease of getting them. HSA is the most abundant plasma protein in humans. Many drugs, including benzodiazepines, bind reversibly to albumin with albumin then acting as a carrier for the drug. This binding can increase the apparent solubility of the drug in the plasma and can influence the distribution, metabolism, and excretion of the drugs. Quenching of albumin fluorescence can be used to study the interactions of these drugs with albumin and characterize the binding affinities and other important binding characteristics. In a preliminary investigation the binding affinities and other binding characteristics for alprazolam, bromazepam, diazepam, flunitrazepam, flurazepam, lorazepam, oxazepam, temazepam, and triazolam to HSA were tabulated. The binding constants of the nine benzodiazepines ranged from $1.14x10^2$ M for diazepam, having the lowest binding affinity, to $8.05x10^6$ M for flunitrazepam, with the highest binding affinity. The binding of these drugs to HSA and the binding affinity of each benzodiazepine derivative may affect the quantitation of these drugs in blood. In the current research, different preparation methods were utilized on samples spiked with known amounts of benzodiazepine. Quantitation was accomplished using an LC/MS/MS method with MRM monitoring which utilized a C18 column and isocratic elution with 0.1% formic acid in Methanol (60%) and 0.1% Formic acid (40%) at a total flow rate of 0.3500mL/min. The temperature range was 40°C-95°C. A comparison of these results will be presented.

"*Discovery-based analyses of various pharmaceuticals in drinking water*" Jordan Stubleski*, Masters Candidate-Forensic Chemistry, Pennsylvania State University, Eberly College of Science, 107 Whitmore, State College, PA 16802; and Frank L. Dorman, Ph.D., Pennsylvania State University, Eberly College of Science, 107 Whitmore, State College, PA 16802

Trace concentrations of pharmaceuticals in drinking water have been detected in a number of different locations. Previous studies have found significant concentrations of a variety of therapeutic groups, including analgesics, anti-inflammatories, statins, psychiatric drugs, and anti-hypertensives, however little research has been conducted on various other groups. For example, the identification and quantification of chemotherapeutics in drinking water has not been significantly reported. In many cases this is likely due to the fact that targeted approaches are almost always used for the analytical determination, when a discovery analysis might have been better advised. Only once a proper discovery analysis has been conducted, can a comprehensive target analysis be developed for complete sample characterization. An additional issue with the analysis of these types of materials in a drinking water matrix is the very low level of expected concentration. This places significant demands on both the sample preparation steps and also on the instrumental analysis.

The objective for this research is to develop a discovery-based characterization method that is capable of detection of a wide variety of therapeutic groups at a level that is appropriate for the analysis of drinking water. This presentation will address the development of a large-volume SPE-based sample preparation

technique that is compatible with both GC and HPLC analyses. The sample extracts will be analyzed using GCxGC-TOFMS and HPLC-TOFMS to make use of the inherent benefits of time-of-flight mass spectrometry in discovery applications. Additionally, the use of the GCxGC technique allows for a much more thorough separation, and also provides a signal-to-noise enhancement to allow for improvements in detectability.

"*SWGTOX Update*"; Jennifer Limoges, NYSP Forensic Investigation Center

"*Uncertainty in Breath Ethanol Testing; Consideration of the Ethanol Blood:Breath Partition Ratio*" Robert H. Powers, Ph.D., DABFT; University of New Haven, West Haven, Connecticut and Connecticut DESPP, Division of Scientific Services, Meriden, CT

Ethanol present in expired breath is commonly utilized as direct evidence of "alcohol impairment", or as an indicator of a blood ethanol concentration from which such impairment may be inferred. Ethanol in the blood is relatively quickly equilibrated with inspired air, presumably in the alveolar space ("air sacs" in the lungs), and that equilibrium is reflected in the "Blood Breath Partition Ratio (BBPR)," relating blood to expired air ethanol concentrations. Ethanol in the blood may then be related to, and determined from ethanol in breath using the BBPR. A strong correlation between blood and breath ethanol concentrations has been demonstrated in many reported data compilations.

BBPR is a individual characteristic, and as a variable in the general population apparently has a mean value of ~ 1:2400. Because of the variation between individuals with respect to BBPR, the resulting uncertainty associated with the inference of a "Whole Blood" ethanol concentration (BAC) from a breath ethanol concentration is considerably larger than the measurement uncertainty associated with only the determination of ethanol in the breath sample itself (a function of gas-phase infrared absorption spectroscopy). Utilization of a 1:2100 BBPR, as is standard practice in the field, (allowing expression of results in terms of g/210 L; with expected equivalence to g/100 ml of blood), results in an underestimation of the true BAC for a majority of individuals (~ 85%) , but results in an overestimation of that value in others (~15%). In the absence of a blood ethanol analysis performed at the same, or at least relatively proximate time that a breath ethanol determination was made, a precise BBPR cannot be determined in any particular case.

With the assumption that BBPR is a normally-distributed variable, a statistical evaluation of BBPR in the general population can be utilized to allow for the determination of a "confidence interval" (CI) in which the true BAC value corresponding to a determined breath ethanol concentration is expected to be. Of primary importance in a legal environment is the low end of such a CI, and whether or not this CI interval overlaps the legal cut-off of 0.08 g/dL. Evaluation of the CI may help ensure that the Forensic Toxicologist presents a defensible opinion in a legal forum, less susceptible to legitimate adversarial challenge. Further, such consideration may help inform prosecutors of reasonable challenge that may be raised to the use of an inferred BAC. Clear understanding of any measured parameter, and its associated uncertainty is of value and potential utility to both sides of our adversarial legal system. Presentation of the Breath Ethanol data in a simple tabular format allows ready determination of the

95% CI associated with any determined breath ethanol value. From a statistical perspective, this same data can be considered as a one-tailed probability consideration, and whether or not the true value for a particular breath ethanol value lies above 0.08 g/210 L can be expressed at the 97% confidence level.

"*A comparison of single versus multi-point calibration for GHB analysis in blood and urine by GCMS*" Samantha Lynes,[1] Vivian Texidor[2] and Robert H. Powers[2], [1]University of New Haven, West Haven, CT; [2]CT DESPP CS/Toxicology Laboratory, Meriden CT; [3]CT DESPP CS/Toxicology Laboratory, Meriden CT

Two different approaches to calibration of GCMS are routinely employed in forensic toxicological analyses. Single point calibration utilizes a single calibrator (or less commonly, a standard), and defines the calibration "curve" as the ray originating with zero response at zero analyte concentration, and passes through the signal/concentration point corresponding to the calibrator or standard. In contrast, multi-point calibration, defines the standard curve as the best fit line (usually linear) generated by three or more calibrators or standards. Commonly, multiple controls are utilized with "single point" calibrations, and single controls with multipoint calibrations. Procedurally, there is usually no difference between calibrators and controls, while there usually is a difference in either the source standard materials from which they are prepared, or preparation is by different analysts in each batch. Either approach provides a measure of procedural reliability.

We have hypothesized that for some GCMS procedures, there is no practical difference between single point calibration with multiple controls, and multi-point calibration with single control, when utilized in "linear" regions of the calibration curve. We have utilized a GHB analytical procedure to test this hypothesis. The experiment consisted of recalculation of GHB batch calibrator and control data, considering the two to be equivalent for the purposes of this comparison. Each batch consisted of a calibrator (20 mg/L GHB) and four controls; 5 mg/L, 10 mg/L, 20 mg/L, and 40 mg/L. Ethylene glycol was used as the internal standard. Batch processing was performed for each analysis by recording the areas of ethylene glycol (m/z 191) and GHB (m/z 233) and calculation of the analyte/I.S. area. Control response was considered as the measureable variable.

Blood and urine sample batches exhibited regions of non-linearity in the GHB concentration range studied, recognizable both visually, and by a progressively increasing departure from expected control values. Both single-point and multi-point calibration approaches were readily able to demonstrate both the linear and non-linear regions. In the "linear region" of both calibrations for urine samples, there was no significant difference in the quantitative accuracy provided by using a single calibrator (evaluated by multiple controls), versus a multi-point calibration (evaluated with a regression coefficient and a single control). In blood samples however, the presence of endogenous GHB introduced systematic error, of greatest magnitude at the low concentration end of the standard curve. Forcing the standard curve through (0,0), which is an inherent part of single-point calibration, does not address this problem. In contrast, multi-point calibration, with the curve not forced through the origin, provides the best performance in such a situation.

In summary, single point calibration with multiple controls can be a valid basis for quantitative analysis, however, distortion of the standard curve by background levels of the target analyte should be considered as a potential uncertainty-introducing factor for any analytical batch.

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"*Zolpidem Metabolites in Urine: An Important Application for Drug-Facilitated Assault Cases*" Laura M. Labay, Joseph Homan, Annette Ervin and Matthew M. McMullin (All authors are affiliated with NMS Labs, Willow Grove, PA)

Zolpidem is a medication primarily prescribed for the short-term treatment of insomnia. Because of its sedative-hypnotic properties the drug has often been implicated in drug-facilitated assault (DFA) cases. It, therefore, stands to reason that when it is suspected an individual has been surreptitiously provided a drug that toxicological testing includes a check for zolpidem. Urine is the matrix type of choice when investigating a crime of this type in that it affords a longer window of detection as compared to blood. One important consideration, however, is that zolpidem undergoes extensive metabolism with only small amounts of parent drug excreted in the urine. Because of this, it is relevant to test for zolpidem's metabolites in urine especially if several days have passed.

At NMS Labs, an in-house study was performed to substantiate that testing was being designed to capture the relevant zolpidem metabolites at suitable concentrations. A male, who is prescribed zolpidem, consumed a single 10 mg dose after control urine was collected at time zero. Over the next several days urine samples were collected and analyzed for zolpidem and two metabolites, zolpidem phenyl-4-carboxylic acid (ZPC) and zolpidem 6-carboxylic acid (ZCA). Prior to this zolpidem use, the drug had not been used for several months. The results demonstrated that by the 24-hour mark the zolpidem concentration fell below 1 ng/mL. In contrast, the metabolites were detectable for approximately 72 hours after use.

Method validation yielded an analytical procedure for the detection of zolpidem, ZPC and ZCA in urine. Briefly, 0.2 mL of urine is mixed with deuterated internal standards (Zolpidem-d6, Zolpidem-d6 Phenyl-4-carboxylic Acid and Zolpidem-d6 6-Carboxylic Acid) and 1.0 M Acetic Acid. Zolpidem and metabolites are then extracted through a Solid Phase Extraction procedure. Eluents are evaporated to dryness and reconstituted with 50% DI water/50% Mobile Phase. Analysis is achieved using reverse phase HPLC separation with positive-ion electrospray tandem mass spectrometry (LC-MS/MS) for detection and quantitation. Two ion transitions are monitored for each analyte (m/z 308.0 to 235.0, 263.0 for zolpidem, m/z 338.4 to 265.2, 293.3 for ZPC and m/z 338.4 to 265.2, 293.3 for ZCA. The lower limit of quantification is 1.0 ng/mL with a linear range of 1.0 - 200 ng/mL.

In 2012, we analyzed a urine sample that was collected following an alleged sexual assault. This urine sample was submitted to the laboratory so that testing could be performed for an antiparkinson drug. While performing this testing, it was noted that the sample contained zolpidem at a concentration less than the reporting limit of the assay (4.0 ng/mL). When this sample was tested using the method detailed above, zolpidem was found at a reportable concentration of 3.9 ng/mL while both metabolites were present at concentrations greater than 200 ng/mL. This case underscores the need to design methods appropriate to their application. For the testing of DFA cases, assays need to reliably detect low level concentrations and test for the appropriate metabolites in the appropriate matrix type.

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"*Concentrations of Various Cathinones or "Bath Salts" in Blood, Urine, Vitreous Humor or Tissue Samples Taken in Postmortem Investigations*" Jeanne Beno, David Nemeth, Donna Nemeth, Michelle Salamone and Patricia Schantz. Monroe County Office of the Medical Examiner Toxicology Laboratory, 740 East Henrietta Road, Rochester NY 14623

The identification and quantification of substituted cathinones or "bath salts" and the interpretation of those analytical findings currently poses a significant challenge for forensic toxicology laboratories. We present our findings regarding these drugs in multiple postmortem cases. The cathinones were initially isolated by solid-phase extraction and analyzed in our GC/MS organic base screen. Tentative identifications were made using the Cayman or SWGDRUG libraries. Confirmation and quantification of drug levels in blood, urine, vitreous humor or tissues were performed by LC/MS/MS once reference standards were obtained. Toxicology results, brief case histories and cause and manner of death from 3 postmortem cases positive for 2-pyrrolidinovalerophenone (2-PVP), 2 cases positive for 3,4-methylenedioxy-pyrovalerone (3,4-MDPV), one case positive for 4-methylethcathinone (4-MEC) and one case positive for 4-methylmethcathinone (mephedrone) will be included.

"*Has Anyone Here Seen Molly?*" James Wesley, Monroe County Crime Laboratory, Rochester, NY

The second wave of synthetics has begun with the introduction of new compounds ranging from Phenylpiperazines to Ketamine and PCP analogs and others. These compounds pose an identification challenge to toxicology laboratories which will require an active communication with their local forensic drug identification lab. To maintain sales momentum (U.S. synthetic drug sales $7B in 2011) manufacturers will attempt to avoid current and future laws by introducing both natural and synthetic compounds structurally dissimilar to cathinones and cannabinoids. We begin with a brief history of Deliriants including tropane alkaloids and incapacitation agents used by the military, and then progress to natural plant based intoxicants, finishing with the current synthetic crisis.

We discuss both availability and identification of these compounds as well as the Serotonin Syndrome, Anticholinergic Syndrome and Excited Delirium in an attempt to match identified drugs to effects.

"*Comprehensive Toxicological Screening using Generic MS/MSALL with SWATH™ Acquisition on the TripleTOF® 5600+ LC/MS/MS System*" Michael Jarvis1, Jesse Seegmiller2, Jenny Moshin2 and Adrian Taylor1 1AB SCIEX, Concord, Canada; 2AB SCIEX, Foster City, USA

Recent advances in Time-of-Flight (TOF) mass spectrometry (MS) have yielded analytical systems with the speed, sensitivity, and dynamic range to be useful for rapid toxicology screening when coupled to a liquid chromatography (LC) system. An additional advantage of screening by LC-TOF-MS is the ability to

perform retrospective data analysis to detect previously unknown compounds. Retrospective data analysis is possible because the experimental approach is generic, and non-targeted.

The use of a Q-TOF tandem mass spectrometer enables the analyst to perform more selective analysis of compounds of interest, by leveraging the enhanced specificity of LC-TOF-MS/MS measurements, thus reducing the possibility of false positives. However, MS/MS measurements traditionally require a targeted experimental approach, since the compounds of interest must be pre-selected for isolation and fragmentation by MS/MS. Due to the targeted nature of such an approach it is not possible to perform retrospective data analysis of MS/MS data to identify previously unknown compounds.

In this work, we present the application of a novel experimental technique employing MS/MS^{ALL} with SWATH™ acquisition for the comprehensive toxicological screening of urine samples, using the TripleTOF® 5600^{+} system. This technique enables the acquisition of MS/MS data throughout the entire LC run for all compounds in a given sample, whether known or unknown, by employing Sequential Windowed Acquisition of all Theoretical masses. Using this approach, high-resolution extracted ion chromatograms can be monitored for the characteristic MS/MS fragment ions of all compounds of interest. Furthermore, retrospective data analysis may be performed to query the data for the presence of any previously unknown compounds, since the acquisition is non-targeted in nature.

A comparison of MS/MS^{ALL} with SWATH™ acquisition versus (i) LC-TOF-MS, and (ii) targeted LC-TOF-MS/MS will be presented. As we will discuss, the major advantages of this technique include: enhanced selectivity, with a reduced occurrence of false positives; the possibility of retrospective data analysis to identify previously unknown compounds; no resultant increase in experimental cycle time as the number of compounds of interest increases.

"*Broad Based Screening of Bath Salts, Synthetic Cannabinoids, and Other Designer Drugs by LC-QQQ-MS and LC-QTOF-MS*"
Anthony P. DeCaprio, Ph.D., DABT, Florida International University, Sponsored by Agilent Technologies, Inc

Northeastern Association of Forensic Scientists

Poster Session Abstracts

"*Effects of blood depth and pool edge geometry on impact spatter patterns*" Daniella Mendez[1], Dan Zamzow, M.S.[2], David Baldwin, Ph.D.[2], [1]Department of Chemical and Physical Sciences, Cedar Crest College, Allentown, PA 18104 [2]Ames Laboratory/Iowa State University, Ames, IA 50011

Bloodstain pattern analysis (BPA) can contain critical information about what has occurred at a crime scene. Understanding the mechanisms of how blood droplets form from impact spatter can help to interpret patterns at a scene. The objective of the research conducted was to test variables causing impact spatter in order to understand the mechanisms of blood droplet formation. Other experimental parameters were held constant. The blood on the wells and flat plates were impacted with the same velocity and from the same dropping height. The variables that were tested were blood pool depth and pool edge geometry. The depth experiments used four large well plates of varying depths. The pool edge geometry was investigated using flat plates; three diameters were used and varying amounts of blood were applied to the plates. The bloodstained targets were analyzed to determine the number, size distribution, and the location of the stains. The results for the depth experiments show that the deeper well plates had larger average stain sizes. The pool edge geometry results show variations in size distributions and angular distributions of stains, where impact of the blood droplet having the largest depth results in stains having the smallest impact angle at the target.

"*The Determination of a Total Uncertainty Budget for the Measurement of Ethanol in Whole Blood*" Amie Dooley, Julia Diaz, Timothy Hahn, Joseph Avella. Nassau County Medical Examiner s Office Department of Forensic Toxicology
adooley@nassaucountyny.gov

An uncertainty budget for the determination of blood alcohol concentration (BAC) by headspace gas chromatography (GC/HS) was calculated using a combination of modeling and empirical approaches. The method employs the use of matrix match blood calibrators ranging in concentration from 0.0157-0.6312 . These are used to calibrate a gas chromatograph equipped with dual column technology. The contributing factors associated with the calculation of the total uncertainty budget were classified as Type A and Type B components, in accordance with the ISO Guide to Expression of Uncertainty. These factors included; the preparation of the calibrators (uCAL), the pooled uncertainty of the repeated measurements of

certified reference control material (uREF), uncertainty in the construction of a linear regression curve (uLIN), and the uncertainty associated with the blood water content of patient specimens (uSP).

Type A uncertainty was calculated using data compiled from 3 control levels analyzed over a 6 month time period (n_1 232, n_2 270, n_3 229). To ensure the estimate was valid across the range of results, the coefficient of variation (4.33, 4.16, 3.62 ; level 1-3 respectively) at each control level was determined and included in a single pooled value (uREF 2.86). Type A uncertainty associated with regression analysis of the calibration curve (uLIN 0.0011) was determined through an analysis of residuals calculated by repeated calibrator measurements (n 73), which was then used to determine a prediction interval or the uncertainty of a calculated value bases upon a best fit curve.

Type B uncertainty related to variation in calibrator preparation (uCAL 0.00214) was comprised of the following uncertainty components: the purity of the stock ethanol solution (uPEtOH 0.00011), temperature, expressed as the thermal expansion coefficient of ethanol (uTemp 0.034 mL), the density of the ethanol (uDEtOH 0.00057 g/mL) and the uncertainty associated with the pipettes (uPip; 0.0122 mL) and flasks (uFlask 0.1224 mL) used during calibrator preparation. Type B uncertainty also included the variation due to differences in the water content of patient specimens (uSP 1.28) in comparison to the matrix matched calibrators.

The total uncertainty of the measurement of blood alcohol content (uBAC) was determined to be 3.13 . When expanded to a 99.7 confidence interval (k 3) the uncertainty budget was found to be 9.42 . To validate the calculated uncertainty budget, blood ethanol proficiency results were examined (n 58). The percentage difference of the lab s reported result to the inter-laboratory mean was calculated. All reported ethanol results demonstrated a percentage difference within the calculated 9.42 uncertainty budget range. The determined budget satisfies accreditory requirements for measurement uncertainty and is suitable for application to forensic inquires where an objective estimation of uncertainty is needed.

"P*ost-Mortem Diagnosis of Chronic Alcohol Abuse using Carbohydrate Deficient Transferrin (CDT)*" Anthula V.Vandoros[1,2], Timothy M Palmbach, Anna Bertaso[2], Federica Bortolotti[2], Franco Tagliaro[2], [1]Dept. of Public Health and Community Medicine, Unit of Forensic Medicine, University of Verona, Verona, Italy, [2]Dept of Forensic Science, University of New Haven, West Haven, CT, USA

Introduction and Aim. Post-mortem data on chronic alcohol abuse can be difficult to obtain due to putrefaction, inadequate background information at time of autopsy, and lack of distinctive pathological characteristics. However, diagnosis of chronic alcohol abuse can aid in death investigations and assist in establishing the cause and the manner of death. A well established biomarker of chronic alcohol abuse is Carbohydrate-Deficient Transferrin (CDT) [1]. Transferrin (Tf) is an iron transport serum glycoprotein consisting of two oligosaccharide chains. CDT is a subset of hypoglycosylated isoforms typically losing one or both glycan chains. Consuming 50-80 grams of alcohol a day for at least 7 days increases CDT serum values. After abstinence CDT returns to normal values in about 2-3 weeks. According to a sound body of literature, the diagnostic sensitivity of this marker ranges from 70-80 , while diagnostic specificity is about 100 . CDT value is expressed as percentage ratio of total transferring; in non alcohol abusers being below 2 [1, 2]. Notwithstanding CDT is a worldwide recognized marker of chronic alcohol abuse, few

studies are published on post-mortem applications largely because of the analytical problems caused by the post-mortem blood. The aim of this work was to investigate the possible use of this marker in post-mortem cases, developing an analytical method based on capillary zone electrophoresis suitable for the analysis of post-mortem samples.

Materials and Methods. 276 post-mortem blood samples from the Office of the Chief Medical Examiner (OCME) in Connecticut were obtained with no restrictions. Measurement of CDT was done by capillary zone electrophoresis using a Beckman Coulter ProteomeLab PA 800 using a U detector at 200 nm in the forward direction. All data was analyzed using 32 Karat software version 7.0.

Results and Discussion Of the 276 samples, only 40 samples resulted in interpretable electropherograms. CDT value in these samples ranged from 0.39 to 13.03 (average 2.85, SD 2.83). 18 subjects showed CDT values higher than the established cut-off of 1.8 (used to distinguish between normal subjects and alcohol abusers) suggesting a condition of chronic alcohol abuse. Of these subjects, only 8 showed specific anatomopathological findings, indicating a condition of chronic alcohol abuse. Moreover, 14 subjects indicated a recent alcohol intake as demonstrated by the determination of blood alcohol content (BAC) (range 0.03-0.41). Of these subjects, 12 were above the legal limit of 0.08 .

Conclusions The data obtained thus far showed that CDT determination could provide information on drinking habits in addition to the data provided at autopsy. Future work will focus on improving the sample preparation procedure to increase casework.

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"*Error rate in calculation of sex via skeletal measurements of a population over time*" Rebecca Skaglin, University of New Haven, West Haven, Connecticut

The determination of sex is a pillar in proper identification of human remains. The following study calculates the error rate in sex determination via sexual dimorphic measurements in human crania from different time periods. Non-metric and metric measurements of the cranium, humerus and femur were used to determine if the remains are male or female. The study over different given time periods, will be used to determine if males and females are becoming more or less similar and if there is a need to update sex identification methods. The sample contains German remains from the 19th and 20th century located at the University of Split in Split, Croatia and the Cleveland Museum of Natural History in Cleveland, Ohio.

Data from samples at the Cleveland Museum of Natural History to be collected at a later date.

"*Near Infared Spectroscopy Analysis of Wines for γ-Butyrolactone (GBL)*" Laura Swanhall and Thomas A. Brettell, Ph.D., D-ABC. Forensic Science Program, Department of Chemistry and Physical Sciences Cedar Crest College, 100 College Drive, Allentown, PA 18104

A method has been developed to determine γ-butyrolactone in wine using near infared spectroscopy (NIRS). γ-Butyrolactone (GBL) has been shown to be a natural component in wines and other products. Wines naturally produce this drug during the fermentation process. GBL is commonly used as an industrial solvent and is a List 1 chemical under federal regulation due to it being an immediate precursor of the illegal synthesis of γ-hydroxybutric acid (GHB), a commonly used date-rape drug. Alcoholic beverages are primarily the matrix in which GHB is typically placed in drug-facilitated sexual assaults. While GBL is typically not placed in beverages with criminal intent there is a need to investigate GBL in alcoholic beverages such as wine because it naturally occurs in wine and because of the interconversion of GBL and GHB.

NIRS was used to quantify GBL since it is a nondestructive analytical technique and easy to use. No sample preparation was necessary. The wines could be analyzed directly. Several red and white wines were analyzed and the GBL concentrations were determined by the standard addition method. A calibration curve was linear (R^2 0.9988) through a concentration of 33.7 – 281.7 mg/L. The LOD and LOQ were 10.1 mg/L and mg/L, respectively. The results have shown that red wines have a greater GBL concentration than white wines. This may be due to the differing pH values between red and white wines during preparation. This study should be useful for forensic analysts to help with the interpretation of wine specimens that have been spiked with GHB and/or GBL.

"*Forensic analysis and validation using GC-IR*" Robert Shipman, Vermont Forensic Laboratory, Waterbury, VT

Our lab has validated a GC-IR instrument for forensic drug casework. Coupling a separation technique to an IR detector allows for analysis of mixtures- which are most samples received by a drug lab. The Spectra- Analysis instrument freezes GC effluent to a cryogenic disk to allow IR analysis of solids. Solid IR spectra produce sharp spectral bands that allow differentiation of similar types of compounds. Current work in the area of synthetic cathinoines and cannabinoids has produced complimentary data to MS data.

"*Investigation of Common Color Tests with Synthetic Cathinones*" Shelli Miller and Thomas A. Brettell, Ph.D., D-ABC. Forensic Science Program, Department of Chemistry and Physical Sciences Cedar Crest College, 100 College Drive, Allentown, PA 18104

Within the last two years, a new class of illicit designer drugs has appeared in the United States, namely, the so-called bath salts , chemically known as *β*-keto-phenethylamines. These bath salts have contained a variety of designer drugs such as methcathinone, mephedrone (4-methylmethcathinone), butylone, methylone (3,4-methylenedioxymethcathinone), methedrone (4-methoxymethcathinone), 3,4-methylenedioxypyrovalerone (MDP) and others.

Forensic chemists employ a small list of common presumptive color tests to screen and detect controlled substances. The aim of the present study was to investigate the reaction of a number of synthetic cathinones with these common color tests with the goal to determine what color tests would provide the best protocol to screen this class of designer drugs.

The project involved using some of the most common color tests to observe the reaction with a number of the most commonly seen selected synthetic cathinones. The following color tests were used in the study: Cobalt thiocyanate, Mandelin, Marquis, Mecke, Ferric Chloride, Froede, Simon s, Zwikker, nitric acid, and Chen-Kao. The reactions with the following *β*-keto-phenethylamines with the above color tests were observed in the study: cathinone, methcathinone, mephedrone (4-methylmethcathinone), butylone, methylone (3,4-methylenedioxymethcathinone), methedrone (4-methoxymethcathinone), 3,4-methylenedioxypyrovalerone (MDP), 3-fluoromethcathinone, Flephedrone (4-fluoromethcathinone), *β*-naphyrone, and diethylpropion. Known NIST-traceable analytes (drugs) were used as positive controls for all color reagents and de-ionized water was used as a negative control for all tests. All reactions were recorded photographically in white porcelain spot plates or clear 3-mL test tubes as they occurred.

The results indicate no one color test can be used to effectively screen the *β*-keto-phenethylamines. Several of the other color reagents reacted and produced chromophores with different *β*-keto-phenethylamines.

"*Evaluation of wet vacuum collection versus traditional methods for collection of biological crime scene samples*" David Patlak, B.A.; Amanda Garrett, B.S.; Catherine Grgicak, Ph.D; Amy Brodeur, M.F.S. Boston University School of Medicine, Program in Biomedical Forensic Sciences, Boston, MA

Generally, biological samples are collected from crime scenes using swabbing, cutting, or taping techniques. However, these methods are limited in their abilities to recover diluted, masked, or otherwise invisible stains. Additionally, their targeted nature allows only a small portion of a larger stain to be collected at one time. In this study, a sterile wet vacuum collection system (M- ac , MSI - Bluffdale, UT) was evaluated in its ability to collect small volume blood stains from various substrates. acuuming was compared to swabbing and taping methods currently used in forensic analysis. Samples were collected from porous and nonporous surfaces and the efficacy of each collection method evaluated with a colorimetric presumptive blood test.

To evaluate each collection method, dilutions containing from 0.25*n*l to 25µl human blood were spotted on each substrate (ceramic tile, blue denim, white low-pile carpet), allowed to dry, and collected using various methods. Prior studies have suggested that a double-swabbing technique may maximize the amount of genomic DNA recovered from a surface[1], but in this study, a single-swab method was used in order to maximally concentrate the collected red blood cells. Adhesive tape has been shown to be an effective, non-destructive method for collecting cellular material from various substrates[2] and was included for comparison. During wet vacuum collection, stains were saturated with sterile buffer and suction was applied to the surrounding area, accumulating the buffer in a collection bottle. Collected buffer was then filtered, and phenolphthalein testing, which has been shown to be an optimal presumptive blood test due to its sensitivity and specificity[3], was performed. Each sample was photographed under constant conditions in order to determine signal intensity.

It was shown that direct methods of sample collection (swabbing, taping) may be more effective than wet vacuuming on nonporous surfaces, but less effective on fibrous surfaces such as denim and carpet. Preliminary results show a positive color-change reaction at 0.025µl blood when collected from tile with swabs or tape, compared to a threshold of 0.025µl when vacuuming. However, positive reactions were only observed above 0.25µl on swabs and tape collections from fibrous surfaces, suggesting that vacuuming may be more effective on these substrates. Further digital image processing and analysis will yield

intensity values for each color reaction, allowing for direct comparison between collection techniques. This study demonstrates the potential of a wet vacuuming method as a suitable technique to collect adhered cellular material from substrates in forensic investigations.

"*Improving DNA Evidence Collection via Quantitative Analysis: A Systems Approach*" Amanda Garrett, David Patlak, Amy Brodeur and Catherine Grgicak Boston University School of Medicine, Program in Biomedical Forensic Sciences, 72 E. Concord St, Rm R806, Boston MA 02118

When collecting biological evidence from a crime scene, it is important to determine the most effective and robust collection method to ensure maximum DNA recovery. Some common biological collection methods include swabbing, cutting, scraping, and taping. Although these common techniques for biological collection have been a mainstay of forensic analysis, each of the methods have significant drawbacks related to them, which include but are not limited to, the lack of surface area that may be processed, possible co-elution of PCR inhibitors and non-optimized elution of cells from the substrate into solution. Therefore, a technique designed to optimize biological collection from items of interest, particularly large items, is necessary and not currently available for forensic use.

The field of pathogen testing, like forensics, also relies on optimized sampling and collection. Recent work in the field of pathogen testing suggests the use of a wet-vacuum collection system would be a valuable addition to the already established forensic methods of collection.[i]

In this study, traditional biological collection methods, including the double swab method and taping, are compared to the wet-vacuum system (Microbial- ac Systems Inc., Bluffdale, UT) through the collection of different volumes of blood (0.075 – 75 µL) on tile, denim, and carpet. Before comparing each method, whole blood extractions and quantification were performed using the QIAmp Investigator extraction protocol (Qiagen, alencia, CA) and the QuantifilerDuo Quantification Kit (ABI, Carlsbad, CA) using the 7500 Detection System. The appropriate volume of blood was spotted onto the surface of each substrate and dried. The sample was then collected through the use of the double swab method, taping using a 2 x 6 cm^2 piece of B DA Instant Lifters (B DA America Inc., New Bedford, MA) or the wet-vacuum system. Additionally, 0.025 – 25 µL of blood were spotted onto each substrate and collected for presumptive testing. After collection, extraction and quantification procedures were performed. Each sample was analyzed in triplicate.

Results have demonstrated that successful DNA recovery can be obtained when the vacuum system is used on non-porous surfaces, particularly when collecting low volumes of blood (Table 1).
Table 1: Average concentrations of whole blood collected on a non-porous surface using various collection methods (in ng/uL).

	75 µL Blood	7.5 µL Blood	0.75 µL Blood	0.075 µL Blood
Whole Blood	58 (+/- 17)	5 (+/- 2)	0.51 (+/- 0.08)	0.03 (+/- 0.03)
Double Swab	**75 (+/- 14)**	**3 (+/- 3)**	0.16 (+/- 0.08)	0.01 (+/- 0.01)
Tape (B𝕍DA Instant Lifters®)	50 (+/- 28)	1 (+/- 1)	0.1 (+/- 0.1)	**0.02 (+/- 0.02)**
𝕍acuum Collection (M-𝕍ac®)	66 (+/- 7)	**3 (+/- 2)**	**0.2 (+/- 0.1)**	**0.02 (+/- 0.02)**

(2 Standard Deviations)

The double swab technique recovered more DNA than the tape for all samples - except when 0.075 µL was spotted. The M- ac recovered more or equivalent quantities of DNA compared to the double swab and tape method when 7.5 µL of blood was collected. This suggests vacuum collection is a viable alternative to double swab and tape collection techniques – especially in situations where there is a low concentration of biological material and a high surface area. Results that focus on recoveries on porous surfaces will also be presented. With these results, the potential use of a wet-vacuum system as a new biological collection technique will be examined.

[i] Bradley B, Saddler F. Comparisons of Meat Carcass Surface Bacterial Collection Efficiencies Utilizing a Novel Wet- acuum Microbial Sampler and the Sponge Method. Proceedings of 54th Annual Reciprocal Meat Conference; 2001 Indianapolis.

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"*A Scientific Approach into the Investigation of the Locard Exchange Principle*" Sarah Haas*and Thomas A. Brettell, Ph.D., D-ABC. Forensic Science Program, Department of Chemical and Physical Sciences Cedar Crest College, 100 College Drive, Allentown, PA 18104

The Locard Exchange Principle explains that whenever two items come in contact, there is a transfer of trace material. The type and amount of trace evidence exchanged may vary depending on the conditions at a crime scene. For instance, when windows or other glass objects are broken, glass fragments may accumulate on the clothing of the individual who broke the glass and/or other people who are in the vicinity. Several factors, such as the type of glass, contact time and/or type of fabric play an important part in the amount of glass that may be transferred or retained.

The purpose of this study was to investigate some of the factors that may be involved in the transfer of glass fragments from common fabrics. Experiments using different sizes and shapes of glass fragments and other parameters such as the amount of pressure on different fabrics were conducted. Trials at different time intervals were completed with five different weave patterns and varying sizes/shapes of broken glass. The size of the glass particles, along with the types of weave patterns found throughout the article of clothing, affected how much glass transferred. When pressure was applied to fabric containing small glass fragments, the glass became imbedded into the fabric and more glass was found on the article of clothing.

The relationship between surface energy, weave pattern, and the amount of glass transferred was investigated. An equation relating several functions such as surface type and surface energy to the amount of glass transferred on fabric is proposed. It is hoped that the preliminary results of these experiments will initiate other investigators to research the topic further and develop a quantitative relationship for the Locard Exchange Principle.

"*Comparing the Efficiency of Draeger Preliminary Breath Test 6810 with Draeger Alcotest 9510*" Helen Tang, Western New England University, Springfield, MA 01119

For many years, the breath test has helped the United States law enforcement detect individuals driving under the influence and by removing them from the road; they improve the safety of the road for their citizens. This research first introduces

the technology of the Draegar Preliminary Breath Test 6810 and the Draeger Alcotest 9510. Both are used in the state of Massachusetts. However, only the Draeger Alcotest 9510 can be introduced to prove an OUI case in Massachusetts Jurisdiction. Therefore, the objective of this research is to compare the efficiency of the two different breath testing instruments currently used in Massachusetts and the reason why Draeger Alcotest 9510 is admissible in court and not Draegar Preliminary Breath Test 6810.

The Draegar Preliminary Breath Test 6810 uses only fuel cell technology and Draeger Alcotest 9510 uses both infrared and fuel cell technology. The research compares the technology and limitations of both instruments. What differentiates Draegar Preliminary Breath Test 6810, which uses only fuel cell technology from Alcotest 9510 is its ability to detect other organic compounds with similar ethanol structures and is affected by the environment such as perfume and temperature. The research concludes with the Draeger Alcotest 9510 as being the better instrument because of its Infrared detection and does not have the same limitations as Draegar Preliminary Breath Test 6810.

"*Frequency of Somatic Mutations in the STR Region of Human DNA amongst Three Sample Types and Their Effect on Genotyping*" Lizzie Martin, Cedar Crest College, Allentown, PA

The frequency and effect of somatic mutations of Short Tandem Repeats (STRs) used in forensic typing are not known and their existence in loci used in forensic genotyping could have significant implications. If somatic mutations exist in these loci, sources of DNA from various matrices could potentially yield different genotypes. As a consequence, the type of matrix (for instance, saliva, blood, and hair) must be considered when determining the appropriate exemplar for comparison with evidentiary samples. In order to evaluate the impact of somatic mutations on forensic genotyping, three biological matrices of DNA from each of approximately 75 Caucasian participants were genotyped. Blood, hair, and saliva samples were subjected to organic extraction followed by quantitation with the Sigma-Aldrich® SYBR® Green JumpStart™ Taq ReadyMix™ kit using a Qiagen Rotor-Gene 6000 real-time PCR instrument. The samples were then amplified and genotyped using the Life Technologies™ AmpFLSTR® kit on a Life Technologies™ 3500xL Genetic Analyzer. The profiles generated were analyzed with Life Technologies™ GeneMapper® ID-X software. Within the small sample size of less than 100 participants analyzed to date, there has been no occurrence of somatic mutations. The occurrence of these mutations is more likely to be found within a larger population size, as in general more mutations are likely to be found when more samples are analyzed. In the future, evidence of somatic mutations will be verified through sequencing to negate the possibility of the existence of mutations in

primer binding sites used during the amplification process. A frequency determination will then be used to evaluate the findings.

"Comparison and Discrimination of Aged and Laundered Fibers by UV-Vis Microspectrophotometry and Colorimetry" **, Sushana S. Williams, West Virginia University, Morgantown, WV**

Fibers are commonly encountered trace evidence materials that are observed and analyzed in forensic science. The analysis of fibers currently relies upon chromatography, microscopy, spectroscopy, and mass spectrometry. While most features of fibers are easily established, the determination and comparison of one important attribute, color, is complex. Factors such as environmental conditions may play an important role when identifying, analyzing and comparing color between questions and known fiber samples.

While color can be evaluated subjectively, it can also be characterized instrumentally. This property may observe subtle or significant differences when fibers have been exposed to aging and laundering. These changes cannot be adequately characterized without instrumental analysis. To standardize and quantify these changes, the Commission Internationale de l'Eclairage (CIE) color characterization system was used in this project.

For the comparison of color, microscopical examination remains the key tool. The Microspectrophotometry (MSP) instrumentation has become a standard analytical method used for measuring color in trace evidence. It is the preferred and accepted method because it is non-destructive. In this study, ultraviolet-visible Microspectrophotometry (UV-Vis MSP or MSP) in transmittance measurement was used to analyze the kinetics of color on aged and laundered textile fibers. In transmittance microscopy, the transmittance curve of a colored sample is an objective description of its physical characteristics, free from the subjective influence that occurs with the human eye when it perceives color. Therefore, the purpose of this research is to objectively measure color change on aged and laundered textile fibers using colorimetry, and statistically evaluating the data obtained to determine how aging and laundering alter the colorimetric data.

Five colors (green, orange, pink, purple and red) polyester shorts were subjected to short term environmental exposure. This project was separated into two parts: Part 1 focused on understanding the degradation of color in fibers by direct exposure to UV light and Part 2 focused on understanding the degradation of color change in fibers after being laundered and aged. This project attempted to replicate the washing and aging affect in a fiber multiple times to determine if precise color comparisons between the treated and unaltered fiber is possible.

Color changes were observed on fibers subjected to the process of aging and washing. Both treatments affected the degradation of color microscopically in fibers. The artificial aging of fibers was seen to affect the saturation of color more than the gloss of the color. The process treatment of washing and aging was seen to affect the gloss of fiber more than the saturation of color within the fiber. Using colorimetry, statistical methods were able to determined where the changes took place and by how much. Unfortunately, CIELab values were unequally affected between colors such that an overall pattern of degradation could not be calculated.

"*Surface Modification Using 9,10-Diphenylanthracene for Fingerprint Enhancement*" Marcel A. Roberts, Elliot Quinteros, Shoronia Cross, John Jay College of Criminal Justice, NY, NY

Fingerprints have been used in areas such as identification, border security and law enforcement. It is at borders that fingerprints have become an invaluable source of information but is being countered by of the high volumes of people that need to be screened. Daily significantly large amounts of people, who need to be identified, enter the United States; but searching them is problematic logistically. A new method for identification must be created that will not only produce immediate results and information but that will also be cost effective. Using 9, 10 diphenylanthracene (DPA), a fluorescent dye, was used to modify glass, polymer and metal surfaces. A uniformly thin layer of DPA was formed on a surface. When a fingerprint was placed on the modified surface a detailed view of the fingerprint was generated. The print was then enhanced using ultra-violet light, in turn increasing the resolution, such that minutiae may be used for fingerprint comparison. The various experiments done for surface modification resulted in a procedure that produced an enhanced print when compared to other methods used. By far in the experimental process, the procedure which produced the best results was by using a mixture of DPA in deionized water. The mixture was transferred to the surface being modified and the water was left to evaporate over a number of hours. The result was an evenly distributed layer of DPA. The method was applied to all the previously mentioned surfaces. The surface that produced the most enhanced resolution of prints was glass. Although an advantage to using the polymers was that they tended to be less expensive, the enhanced print generated was not as detailed as the glass surfaces. This approach can be combined with other methods of detection to create a system that can identify a person and a substance on their fingers.

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"Determinations of Best Practices for Chemical Enhancement of Two-Dimensional Footwear Impressions" **Heather Moody, Cedar Crest College, Allentown, PA**

Several types of chemical enhancement procedures exist for two-dimensional footwear impressions. This presentation will compare the specificity and sensitivity of five common chemical enhancement methods often employed at crime scenes to enhance two-dimensional footwear impressions made from soil or dust. In this study, two dimensional footwear impressions comprised of dust and wet soil were made on vinyl, industrial floor tiles using the same shoes and then enhanced with either bromophenol blue, potassium thiocyanate, ammonium thiocyanate, 1,8-diazafluoren-9-one, or bromocresol green. Results were obtained with all enhancement reagents tested with the exception of 1,8-diazafluoren-9-one. Dust impressions were tested up to six steps while wet soil prints were made by walking on several tiles, with the fourth, seventh, and tenth step enhanced. The dust impressions enhanced with either potassium thiocyanate or bromophenol blue were compared side by side. The specificity of both impressions was examined by looking for completeness of the shoe's tread design. The toe region of the tread appeared more often than the heel when enhanced with potassium thiocyanate. The impressions enhanced with the bromophenol blue showed the majority of both the heel and toe regions of the tread. Sensitivity was measured by determining the furthest step the reagent could reasonably enhance the impression. Bromophenol blue had very good sensitivity with all six steps showing enhancement. Potassium thiocyanate showed less sensitivity with only steps one through four showing enhancement. The wet soil impressions were enhanced with potassium thiocyanate, ammonium thiocyanate, bromocrescol green, or bromophenol blue, respectively. The tiles enhanced with bromocresol green and bromophenol blue showed the most tread design demonstrating good specificity, while less was seen with the ammonium thiocyanate and potassium thiocyanate. The bromocrescol green showed an equal amount of enhancement across the fourth, seventh, and tenth steps. The bromophenol blue showed good sensitivity with a very slight decrease in enhancement across the descending steps. The ammonium thiocyanate showed approximately the same amount of enhancement on all steps as the potassium thiocyanate. Overall the bromocrescol green and bromophenol blue reagents appear to work best under experimental conditions, while ammonium thiocyanate showed slightly better enhancement than the potassium thiocyanate. Future study will include enhancements with imprints made with different soil or dust types since results could theoretically change based on changing imprint composition.

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"*Preparing for court appearances: teaching forensic students the importance of quality assurance and quality control*" Gianna Mancuso, Dr. Louis Traficante, and Dr. Andrew Dutton; Willow Laboratories, Lynn, MA and Department of Chemistry and Biochemistry, Suffolk University Boston, MA

For a forensic laboratory to be considered reliable in any respect, both quality assurance and quality control must be enforced daily. The importance of quality assurance and quality control are vital in becoming an expert witness as well as a reliable technician, and must be taught in students' undergraduate courses. Quality assurance (QA) is practiced through the proper training of laboratory technicians, through a clear chain of command, and through proper cleaning and calibration of all tools and instruments used. Quality control (QC) is performed by running control samples throughout the day to ensure all instruments are working properly and reading in the correct ranges for the specific samples run on that instrument. Undergraduate professors must better explain QA and QC in their curriculums and help coach students for their inevitable court appearances. QA and QC are the easiest way for lawyers to find errors in the expert witness's report. Lawyers may ask questions about temperature checks, calibration, instrument maintenance, possible sources of error, chain of custody, and technician training. Therefore, early on, students should be familiar with the importance of QA and QC as well as the significance of proper scientific recording. Students should also be familiar with standard operating procedures (SOPs), and the importance of following them and keeping them up to date. Professors should explain what QA and QC are during lecture and enforce what was learned in lecture in the laboratory. Doing this will give students the benefit of knowing why QA and QC are performed as well as how to perform the different tasks. For students who are interested in the forensic science field, the importance of QA and QC should be enforced through the use of mock trials. When preparing for trial, the expert witness reviews all QA and QC data as well as any other pertinent information relating to the case. The expert must know exactly what lawyers are looking for when reviewing the information and should know how to explain that information to the court. Professors can help prepare students by sending out mock subpoenas to each student and coach them through each step of the preparation. Then students can go through several practice runs until they learn how to handle themselves in court. At the end of the semester, the final court appearance will occur when they are given a new subpoena and have to prepare everything on their own. Through this new curriculum, students will be better equipped for working in a forensic laboratory from running daily controls to preparing for trials.

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"*Optimized Automation of the DNA IQ® System for Human STR Identification using the Hamilton STARlet® Forensic Workstation*" Lois Tack, PhD[1], Daniel Hellwig, MSFS[2], Ryan Buchanan, MS, MBA[2], and Robert Sheen[2]; [1]Hamilton Robotics, 4970 Energy Way, Reno NV 89502, lois.tack@hamiltoncompany.com, [2]Sorenson Forensics, 2495 South West Temple, Salt Lake City UT

Implementation of forensic DNA purification methods to produce high quality DNA suitable for today's multiplexed STR (short tandem repeat) analysis procedures has proven challenging for crime labs who also contend with ever increasing processing demands. The DNA IQ® System from Promega is a widely used genomic DNA (gDNA) extraction method for database and casework labs. We describe here how Sorenson Forensics has used the Hamilton MICROLAB® STARlet® workstation to automate the DNA IQ system method to increase throughput while maintaining strict gDNA sample typing performance. The automated DNA IQ method utilizes a magnetic based purification technology involving binding of DNA to silica-coated magnetic particles in the presence of a strong chaotropic salt, washing of the magnetic resin to remove PCR inhibitors, and DNA elution from the resin using low-salt conditions and heated shaking. The walk-away automated gDNA extraction protocol was optimized and validated using different sample types including blood and saliva over a wide range of starting concentrations. Sorenson's validation plan involved analyzing the purified DNA as follows: recovered DNA quantity measured by real time quantitative PCR (qPCR), degree of inhibition as measured using real time IPC (internal positive control), and the genotyping quality of relative STR peak heights. DNA from these samples was free of detectable PCR inhibitors and the resulting short tandem repeat (STR) profiles were complete, conclusive, and lacking PCR artifacts. Sorenson has used the same STARlet hardware system to develop and validate other forensic DNA processing methods, including the PrepFiler DNA Purification Kit (Life Technologies/ABI) and a large variety of qPCR and STR kits combined with DNA normalization/dilution steps.

Robotic liquid handling workstations offer many advantages, including increased throughput, reduced user error, and more reliable data production. However, following robotic installation, re-validation of current manual methodology and new SOP creation is often a barrier to effective implementation of these automation tools. To meet this need, Sorenson Forensics offers robotic validation services to the forensic community. This includes implementation of the DNA IQ system method using the STARlet Forensic Workstation to automate this and a variety of other common forensic DNA isolation and PCR analysis methods for human STR typing. Sorenson Forensics is well known for its comprehensive forensic validation DNA

services to federal, state and local crime laboratories and private industry clients providing expertise and proficiencies in areas such for STR and Y-STR analysis.

"*Still Another Look at the Classification of Acrylic Fibers by FTIR Microscopy*" Laura Schneider and Edward G. Bartick, Forensic Science Program, Department of Chemistry and Biochemistry, Suffolk University, Boston, MA 02014, leschneider@suffolk.edu, ebartick@suffolk.edu, Stephen L. Morgan, Ph.D., Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208, morgansl@mailbox.sc.edu, John V. Goodpaster, Ph.D., Forensic and Investigative Sciences Program, Indiana University, Purdue University, Indianapolis, IN 46202, jvgoodpa.edu@iupui

FTIR analysis of acrylic fibers is of great interest to trace fiber evidence examiners because of the sub-classification that can be elucidated through the spectral interpretation. In 1995 Grieve[1] took a second look at acrylic fiber sub-classification where he identified 20 different chemical types by FTIR. In addition, Tungol, et al[2] considered sub-classification through spectral peak ratios taking into account co-monomers of the acrylic fibers. We have taken a further look to consider additional sub-classification and to produce a statistical basis of questioned and known fiber match criterion and probabilities of being randomly found within the fiber population.

A well characterized collection of commercial acrylic fibers, from known manufactures (We will refer to this as the Suffolk University Acrylic Library (SUAL)) was used to validate the FTIR spectral search effectiveness when using the FBI fiber library that is based on polymeric composition[3]. With fibers of the same known source high scores as high as 99.99% were achieved when searching against this 20 year old FBI library. One hundred eight-five acrylic fibers of unknown sub-generic class from a database of fibers collected at the University of South Carolina (USC) were then successfully searched against both FBI and SUAL libraries. They were identified as 166 polyacrylonitrile/vinyl acetate/sulfonate (PAN/VA/SUL) and 19 polyacrylonitrile/methyl acrylate/sulfonate (PAN/MA/SUL) acrylics.

Ten separate scans of spectra from the SUAL library were then compared with an additional set of ten spectra (group) obtained at a different time by multivariate statistical analysis (MVA) with the application of principle components and linear discriminant Analysis (PCA and LDA) to determine matching capability and scoring criteria. At this time we have been unsuccessful using these methods on the acrylic spectra.

In addition, fibers from manufacturers of methyl acrylate copolymers were analyzed by comparing the ratios of the acrylonitrile versus the carbonyl peaks to attempt to identify the manufacturer and possibly the product line by the ratios measured. The measurements were made by both peak height and area.

Also, the PCA and LDA methods were tested over the range of the nitrile and carbonyl peaks. At this time we have not attained successful results capable of distinguishing the fibers tested.
The work of multivariate analysis and peak ratios is still under investigation. In conclusion, the traditional library searching technique has continued to demonstrate good results when properly used.

"Latent Print Recovery on the Adhesive Side of Pressure-Sensitive Tape using Adhesive Neutralizers"
Valerie Bennett, Suffolk University and Boston Police Latent Prints Section, Boston, MA

Pressure-sensitive adhesive tapes are often submitted as evidence for latent print recovery. For forensic laboratories to be capable of developing any possible ridge detail on the tape's surface, the tape adhesive must first be neutralized, allowing for print preservation during a non-destructive separation from its substrate. Two neutralizing reagents, Un-Stick Adhesive Remover and Tape Release Agent, commercially sold through a forensic supply wholesaler, were tested to determine their potential in latent print recovery with the Boston Police Department Latent Print Section. These reagents were tested to verify their ability to separate tape, their affects on the tape adhesive, the post-application capability of recovering ridge detail on both the tape and its underlying substrate, and the performance benefits and limitations for each technique. The Un-Stick Adhesive Remover and the Tape Release Agent were tested on samples consisting of two pieces of the same type of tape adhered adhesive-to-adhesive as well as adhesive-to-non-adhesive, and on samples of tape adhered to a non-porous surface and to a porous surface. Masking tape, packaging tape, scotch tape, electrical tape, and duct tape were each tested. Primary testing was conducted on ideal samples, which do not approximate actual casework, but represent conditions that would be ideal to test the basic functioning of the techniques. Both adhesive neutralizers yielded results below the threshold acceptable for casework when applied to any sample of duct tape due to excessive adhesive deterioration. Electrical tape, scotch tape, masking tape, and packaging tape, however, were deemed successful under ideal conditions and thus advanced to testing under realistic conditions. Realistic samples were prepared in such a way to closely approximate actual casework. For all tape samples, results from this secondary testing were below the threshold for casework, both in adhesive neutralization, as well as ridge detail recovery. It was concluded that both Un-Stick

Adhesive Remover and Tape Release Agent are unsuitable for casework in the Latent Print Section of the Boston Police Department.

"Breakthrough in forensic workflow automation - AutoLysSTAR® eliminates sample preparation and lysis bottlenecks -validation study at the NFI" **Lois Tack[1], Bas de Jong[2], and Laurent Baron[3]; [1]Hamilton Robotics, 4970 Energy Way, Reno NV 89502, lois.tack@hamiltoncompany.com; [2]The Netherlands Forensic Institute (NFI), Postbus 24044, 2490 AA The Hague, Netherlands; [3]Hamilton Robotics, P.O. Box 26, CH-7402 Bonaduz GR, Switzerland**

Forensic DNA sample processing frequently involves manual lysis and extraction to prepare cleared lysates from various biological samples (swabs, cloth cuttings, etc.) prior to DNA purification. Lysis steps are time-consuming, introduce gaps in sample workflows, loss of traceability and can increase contamination risks and user errors. Sample preparation and lysis are major bottlenecks for many forensic labs.

We describe the AutoLys STAR®, a new walk-away automated lysis solution from Hamilton Robotics and present validation data produced in collaboration with the Netherlands Forensic Institute (NFI). The AutoLys STAR is a single instrument platform that processes four 24-tube racks (96 samples) at once, providing complete sample ID traceability. The AutoLys includes new "smart" spin column tubes plus a unique channel/tool to fully automate capping/decapping spin tubes, tube movement, lift-&-lock of inner tube prior to on-deck centrifugation, and inner tube removal. Separate independent pipettors add extraction buffer prior to on-deck heated/shaking incubations and subsequently transfer cleared lysates to new tubes/plates for DNA purification. Sample purification can be on same deck or transferred for further processing on other instruments. AutoLys can run overnight – which is great for labs with backlogs. We will also show analysis data comparing AutoLys lysis with manual processing. DNA yields are comparable or improved, while maintaining DNA quality with reduced error and contamination and full sample traceability– all leading to more successful STR profiling.

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"*Analysis of Erectile Dysfunction Drugs and their Analogs*" Susan Neith, Forensic Chemist, NMS Labs, Willow Grove, Pennsylvania

Today there is an ever changing movement in illicit drug manufacturing. New drug analogs and synthetic drugs are being created at an alarming rate and are easily dispensed to the general public through the internet.

Drug such as Sildenafil (Viagra), vardenafil (Levitra) and tadalifil (Cialis) were FDA approved for the treatment of erectile dysfunction. Herbal substances claiming to have the same effects as viagra have emerged and are predominantly sold through the internet however they usually do not contain sildenafil citrate. Synthetic chemical compounds similar to sildenafil have been found as adulterants in many of these supplements which are sold as herbal viagra or "natural" sexual enhancement products. Most are listed as 100% safe and natural, free from side effects and no prescription needed.

Analogs of erectile dysfunction drugs often carry a number of dangerous side effects. Also, patent infringement suits have been filed by pharmaceutical companies against the manufacturers of these products.

There is a need for laboratories to keep up with current drug trends and have the ability to detect and identify these new substances as they emerge. Drug/chemical libraries also need to be updated regularly to help in the identification of these substances.

High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) can still be valuable tools in identifying substances that may not be easily differentiated by Gas Chromatography / Mass Spectrometry (GC/MS). These different analytical techniques can be used to confirm the presence of these substances. Data will be presented using a combination of the above referenced techniques.

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"*Increased PCR Cycles, Post-PCR Purification, and Their Effect on Low Copy Number DNA Profiles*"

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Low Copy Number (LCN) DNA analysis has many benefits. It can lead to bone samples providing improved matching statistics over mtDNA, as well as allow samples with low amounts of template DNA taken from crime scenes to reveal relevant information about a crime. However, under standard DNA profiling procedures, LCN DNA is not likely to produce profiles of good quality. Luckily, procedures for increasing the sensitivity of DNA testing and profiling have been suggested and are currently being tested. Increasing the PCR cycles allows for more copies of target DNA to be created. Therefore, the LCN DNA is more likely to be detected and could produce a more complete and useful profile. Post-PCR purification involves the filtering of DNA after the standard number of PCR cycles (usually 28). This process removes leftover components of the PCR reaction (such as nucleotides) and concentrates the amplified DNA. The lack of electrically active components and the concentrated DNA makes it more likely that the target DNA will be injected in to the DNA sequencer, hopefully producing a more complete profile. In this experiment, these two methods (increased cycle number and Post-PCR purification) were compared to determine which was more effective for producing imporved LCN DNA profiles.

For this experiment, LCN DNA was defined as samples containing less tha 100pg of DNA. Therefore, the isolated DNA from our subjects was serially diluted to concentrations ranging from 100 pg to 1 pg. These titrations were then subjected to the various methods mentioned above including sets that ran for 28 cycles or 34 cycles, as well as sets which did and did not undergo Post-PCR purification. These samples were then analyzed on a DNA sequencer and their profiles were detected. The profiles were then compared for profile completeness compared to knowns, peak height compared to knowns, and the presence of any unwanted anomalies noted.

Thus far, two sets of titrations were compared, both having undergone Post-PCR purification. One set had been submitted to 28 PCR cycles and the other 34. The amount of correct alleles obtained from the titrations ranged from 0 to 13 in the 28 cycle set, and 1 to 13 in the 34 cycle set. From the 28 cycle set, the optimum amount of correct alleles, when compared to a known profile, was created from the 97.0 pg titration and was found to be 13 correct alleles. The optimum amount of correct alleles from the 34 titration only produced 8 correct alleles. From the 28 cycle set, the average peak height at each locus, for each titration, ranged from 0 to 162. In comparison, the average peak heights from the 34 cycle set were much higher,

ranging from 0 to 8047. This suggest that the addition of 6 cycles does allow for a higher detectability in the DNA resulting in more complete profiles at lower concentrations. Many of the anomalies noted in previously published studies were also present in this study. Allele drop out was present and accounted for much of the imcompleteness of the profiles. Allele drop in was not noticed, suggesting that procedures to prevent contamination were working well. Binning accuracy issues were noted in the 34 cycle set. This was probably caused by the high detection levels present, causing some of the correct alleles to go off scale and not be detected of binned accurately due to peak width. Additional data for this study are forth coming, such as the effect of Post-PCR purification alone and a more in depth analysis of the anomalies created by both methods.

"*Cathinone Related Drugs ("Bath Salts") in Postmortem Cases and Driving Under the Influence of Drug (DUID) Cases*" Jeanne Beno, David J. Nemeth, Donna Nemeth, Michelle Salamone, Patricia Schantz Monroe County Office of the Medical Examiner Toxicology Laboratory, 740 East Henrietta Road, Rochester, NY 14623

In the past two years, our laboratory has seen "bath salts" or cathinone derivatives appear in both our postmortem and driving under the influence of drugs (DUID) cases. We present the data for 15 cases (5 postmortem and 10 DUID) which contain 2-pyrrolidinovalerophenone (2-PVP), 4-methylethcathinone, 4-methylmethcathinone (mephedrone), 3,4-methylenedioxypyrovalerone (3,4-MDPV) or methylone. We will discuss the challenges of identifying these structurally similar compounds using solid phase extraction (SPE) followed by gas chromatography/mass spectrometry (GCMS) and library matching. A targeted bath salt drug screen using liquid chromatography/mass spectrometry/mass spectrometry (LCMSMS) has also been constructed. Finally, SPE and LCMSMS analytical methods used to confirm and obtain quantitative data will be shown.

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