Northeastern Association of Forensic Scientists

PROCEEDINGS

of the Northeastern Association of Forensic Scientists

September 2013

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Northeastern Association of Forensic Scientists

Poster Abstracts

***An Animal Model Study of the Pharmacology of Designer Cathinones and Cannabinoids**

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Abstract:

The incidence of designer drugs such as synthetic cathinones and cannabinoids has dramatically increased despite the changes in laws regulating the spread of these drugs. The minimal understanding of these drugs drives the general public in obtaining these chemicals especially as they are often sold as legal alternatives to illicit drugs. Synthetic cathinones are central nervous system stimulants that have been reported to have effects similar to amphetamines and cocaine. Synthetic cannabinoids are reported to be cannabinoid receptor agonists. As a consequence of the ever-changing chemical structures of these compounds, no detailed toxicological and pharmacological studies have been reported in literature. The complexity of the emerging drugs has placed a burden on the medical and forensic community in determining the toxicity and adverse effects of these drugs. The poster describes the use of planarian flatworms as simple animal models for the study of novel cathinones and cannabinoids. Planarians are well-established in literature as animal models used in pharmacological studies of psychoactive drugs. These worms are useful because of their similarities to vertebrate nervous systems. The poster discusses the potential of using this animal model to study various pharmacological effects of emerging designer drugs combinations. The study will allow us to understand the effects by studying the motility and behavior of the worms in water habitats after being administered to the drugs of interest. They exhibit specific behavioral responses to drugs and these responses can be visually recorded and quantified with dose-response curves. The ease of this model provides opportunities to screen several drugs with the possibility of screening testing suspect materials for psychoactive substances based on the behavioral response observed in the flatworms

***Collegiate Competition**

A Comprehensive Solution for Quantitation of Ketamine and Ketamine Metabolites in Urine Using LC-MS/MS and GC-MS/MS

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Abstract:

Ketamine is a dissociative anesthetic at sub dose, which has been prohibited from use in human and animals. It generates hallucination, arousal feeling, and has been abused as a “club drug” on the street. As the increase of popularity of ketamine worldwide, the need for fast screening and quantitation of ketamine continuously grows. Mass spectrometry couple with gas chromatography (GC-MS) is widely used in forensic and toxicology. GC-MS selective ion monitoring (SIM) mode is powerful in forensic cases, and has been used for ketamine quantitation. Later LC-tandem mass spectrometry (LC-MS/MS) is emerging as an alternative of GC-MS in forensic laboratories. With LC-MS/MS platform, certain matrix samples, such as urine, can be even directly

introduced, therefore significantly simplifies sample preparation. Nevertheless, GC-MS is still an indispensable tool. With the introduction of GC-MS/MS, the selectivity and sensitivity is much improved and serves as a complementary tool to LC-MS/MS technique.

In the current work, tandem mass spectrometry method for quantitation of ketamine and its metabolites norketamine (NK), dehydronorketamine (DHNK) in urine was developed on Bruker LC-MS/MS and GC-MS/MS system respectively. The LC-MS/MS method employs "dilute-and-shoot" strategy, that urine samples were diluted and directly introduced to the LC-MS/MS platform. "Dilute-and-shoot" LC-MS strategy for ketamine quantitation in urine features simple sample preparation without losing method sensitivity and robustness. Quantitation is determined from 1 – 1000 ng/mL for all three drugs, sufficient for practical forensic and toxicology application and the detection limit is achieved in the sub pg/mL level. The LC-MS/MS system demonstrates excellent reproducibility with multiple injections of heavy matrix. At low ng/mL level, good robustness is exhibited as well. Alternatively, GC-MS/MS method for quantification of ketamine and its metabolites in urine was demonstrated. Sample extraction, such as solid phase extraction (SPE) has to be implemented prior to GC-MS/MS analysis; on the other side it serves as a concentration step to compensate the smaller injection volume.

A SPE C18 Tip was employed for quick exaction within in a 5min. Using GC-MS/MS analysis, the LOQ for ketamine, NK and DHNK is 0.5, 1 and 10 ng/mL respectively, showing good sensitivity. This work demonstrates a good example that LC-MS/MS and GC-MS/MS platforms are both feasible technologies for ketamine quantitation in urine matrix, providing alternative options at limited instrumentation situations.

Effect of Different Cotton Fabrics on Bloodstain Drying Time

Sachie Marubayashi and William Welsh - Drexel University

Abstract:

Bloodstain pattern analysis is important for the reconstruction of events from a crime. While recent studies have examined the effect of different surfaces and fabric compositions on bloodstain patterns, detailed analysis of specific materials have yet to be determined. In this study we wish to expand on the previous research and perform a more exhaustive analysis of blood drying times on textiles. To this end we included 100% cotton fabrics of varying weave and thread count. The fabrics include T-shirt material, A-shirts, jeans, pillow covering, and linens of differing thread count; all of which have been through the wash/dry cycle at least three times. We focus on the effect of blood drop height, volume, and drop angle on these different fabrics to determine a relation with blood drying time. We believe that with tighter weave and higher thread count, greater blood volume will increase drying time. Additionally, we believe increased drop height and angle will decrease drying time due to increased surface area exposure allowing for faster evaporation. Drying time will be determined by the alteration of weight over time incorporating the issue of organic residual matter. Each combination of conditions will be performed in replicate to analyze the average weight change as well as the rate of change. Additionally, ANOVA analysis will be performed to determine the relationship between the different variables. Initial replicate testing on T-shirt and jean fabric types under controlled conditions demonstrate that this method is reliable and consistent. We believe our results to be important for further analysis of fabric influence on bloodstain drying time. This knowledge will help in crime scene analysis and reconstruction in the future.

***Investigation of the Stoichiometry in the Modified Ferric Hydroxamate Test for Gamma-butyrolactone**

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Abstract:

Alston and Ng developed a color test for the detection of gamma-hydroxybutyric acid (GHB) using a modified ferric hydroxamate color test for lactones.[1] Michalik and Brettell later modified the test into an effective spot test for GHB.[2] The procedure consists of the following steps: 1) GHB is converted into its lactone form, GBL, by the addition of concentrated sulfuric acid; 2) Addition of hydroxylamine hydrochloride in 95% ethanol and 6M potassium hydroxide forms an intermediate; 3) Addition of iron (III) chloride forms the final colored-complex after a pH adjustment to 3 using concentrated hydrochloric acid. Alston and Ng proposed a 3:1 ratio for GBL and Fe^{3+}; however, no information was given in the report for the determination of the stoichiometry.[1]

The goal of this project was to use the method of continuous variation to determine the stoichiometry of the purple-colored complex that forms with iron in the presence of GBL. For research purposes, it was assumed that the conversion of GHB to GBL is 100%; thus, GBL was used as the starting material. 0.2 mL increments from 0.0 - 2.0 mL of 0.031M GBL was transferred to separate 10 mL beakers. 2 mL 0.5 M hydroxylamine hydrochloride in 95% ethanol and 1 mL 6M sodium hydroxide were then quantitatively transferred to each beaker. Then 0.2 mL increments from 2.0 - 0.0 mL of fresh 0.031M iron (III) chloride solution were transferred to each beaker, respectively. This resulted in the total mole fraction of GBL and Fe^{3+} being held constant during this procedure. Spectra were collected on a DU-800 UV-Vis spectrophotometer in the spectral range of 350 – 800 nm at a scan rate of 1200 nm/min. Absorbance values were collected at 500 nm (λ_{MAX} for the colored complex) and a Job plot was created. Five replicates of each sample were analyzed to develop the Job plot. A second trial, also with five replicates, substituted 6M sulfuric acid for 6M hydrochloric acid since Alston and Ng used sulfuric acid in their assay.[1]

The Job plots with the hydrochloric acid indicated the stoichiometry of the GBL/hydroxamate-Fe^{3+} complex is actually 1:1, not 3:1 as suggested by Alston and Ng (1). The maximum absorbance of each Job plot was located at a 0.5 mole fraction (GBL/GBL and Fe^{3+}), where there were equal moles of GBL and Fe^{3+}. When the Job plots with the sulfuric acid were created, the trend also indicated that the stoichiometry of the GBL/hydroxamate-Fe^{3+} complex is 1:1. However, the overall absorbance values were lower for the sulfuric acid trials than they were for the hydrochloric acid trials, suggesting that the molar absorptivity of the complex formed using hydrochloric acid is higher than that of the complex formed using sulfuric acid.

In conclusion, these experiments indicate that the stoichiometry of the GBL-Fe^{3+} complex formed in the modified ferric hydroxamate test is actually 1:1, not 3:1 as previously reported by Alston and Ng[1]. Studies using both hydrochloric acid and sulfuric acid corroborated that finding.

*** Collegiate Competition**

Does Firearms Chemical Testing Interfere With DNA Testing?

Kristin Alfano - University of New Haven
Wesley Krupp and Joseph Galdi - Suffolk County Crime Lab

Abstract:

The purpose of this study was to determine if the chemicals used for distance determination of gun shots would interfere with DNA analysis. A known blood sample was placed on four separate pieces of white cotton. Three of the cotton pieces were subjected to one of each of the chemical tests conducted by the Firearms Section (Modified Griess, Dithiooxamide and Sodium Rhodizonate). The fourth sample was subjected to all three tests. Following the chemical testing, a portion of each blood stain was cut out for DNA analysis. Expected results were obtained from each sample with no decrease in the yield of DNA obtained. Based on this study, distance determination analysis may be performed on evidence prior to sampling for DNA.

The Million Dollar Forgery

Greg Kettering and Lisa Ragaza M.S. CT Division of Scientific Services, Department of Emergency Services and Public Protection.

Abstract:

Massachusetts State Police requested assistance with a forgery case involving a disputed property claim worth millions of dollars. Signatures and forms were allegedly forged and the property was claimed by the suspect.

The case was delivered to the Division of Scientific Services for signature and raised seal analysis involving a notary stamp that was made in the early 1980s and confiscated from the home of the suspect. The examiners travelled to Crystal Rock, Inc., the company that actually made the stamp, and discovered unique characteristics that could be associated with the stamp which were made during the manufacturing process.

In addition, there was damage to the notary stamp which was visible on the stamp, on test impressions made from the stamp, and on the document in question. Individual characteristics associated with notary stamp along with signature examinations that implicated the suspect were made. The case went to trial and the suspect was found guilty of forgery.

Establishing a Method to Determine the Statistical Value of Fiber Evidence

Laura Pritchard, Research Advisor - Dr. John A. Reffner John Jay College of Criminal Justice

Abstract:

This project was done to establish a method in determining the statistical value of fiber evidence. Fibers are ubiquitous and therefore are a common piece of trace evidence collected at crime scenes. In order to identify these fibers, characteristics have been found to differentiate between manmade and natural fibers. Numerous population studies have been done to provide fiber analysts with values of what fibers are out there, but variation

arises based on geographical location and seasonal weather. Target studies have also been completed to show the chance of finding specific fibers in a random population of fibers. With an increase amount of fibers in the world and variation based on location, there has been difficulty establishing a reliable database that incorporates all of the fibers in the world. This study was done to show that each crime scene can be unique and a collection of fibers from that scene can provide the analyst with a closed population to compare the suspect fiber to. Dryer lint from 14 dryers was collected. A sample from each was mounted on its own microscope slide and then analyzed with a polarized light microscope. Samples were viewed in 3 increments of 100 for 300 total fibers from each slide. A worksheet was formulated to efficiently tally up the color, cross sectional shape, parallel refractive index, perpendicular refractive index, dichroism, and birefringence of each fiber in the sample sets. This data was then analyzed using the Fisher's exact test for count data to calculate the independence of one sample to the other, showing whether or not samples can be similar to one another.

***The Identification of Controlled Substances by TLC-SERS**

Kasey Cargill and Dr. Brooke Kammrath - University of New Haven

Abstract:

This research project evaluated the method of thin layer chromatography combined with surface-enhanced Raman spectroscopy (TLC-SERS) for the purpose of separating and identifying controlled substances. This combined technique has the potential to benefit the forensic science community because it requires less sample, time, and money when compared to other methods of analysis in addition to adhering to the standards for positive drug identification established by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG).

TLC is a method of separation that is commonly used as a screening tool in forensic laboratories because it is quick, inexpensive, and efficient for separating components of mixtures. However, TLC is only used for preliminary identifications, and in order to make a positive identification of a sample, a confirmatory method is required. TLC-SERS enables this confirmation to be made directly on the TLC plate, because SERS can be performed on the separated TLC spots. SERS is an analytical method that can enhance Raman scattering via the interaction of a metallic nanoparticle with certain chemicals, and has been shown in the literature to be useful for the identification of controlled substances. In addition, SERS can correct for the two main disadvantages of normal Raman spectroscopy: low sensitivity and fluorescence. SERS can be completed on the TLC plate by adding a metallic colloid to a separated TLC spot, and then directly analyzing with a Raman spectrometer. SERS spectra are unique to single compounds and therefore allow this method to be used as a technique for identification.

The illicit drugs cocaine, methamphetamine, MDMA, and codeine were analyzed using TLC-SERS, which proved to be a successful method of separation and identification. TLC-SERS, when compared to other methods of drug identification, allows for a minimized amount of required sample to be tested, a reduced cost for analysis, and decreased total analysis time for complete SWGDRUG approved drug identification.

*** Collegiate Competition**

The Body Bronzer Case - A Multidisciplinary Approach to the Examination of Physical Evidence from a Sexual Assualt

Karen Lamy, M.S., Christine Roy, M.S., Maria Warner, M.S.
Jack Hubball Ph.D.- CT Division of Scientific Services, Department of Emergency Serives and Public Protection

Abstract:

Physical evidence from a sexual assault case that occurred in October of 2008 was examined by personnel from the Forensic Biology, Chemistry, Trace, and DNA sections of the CT Division of Scientific

Services. A female victim was sexually assaulted (vaginal and anal) by a masked assailant who covered the victim's face with a sheet during the entire assault. The sexual assault occurred in the presence of the victim's seven month old daughter. After the sexual assault, the assailant dragged the victim from her bedroom to the kitchen where he taped her to a chair. The victim recognized the suspect's voice to be that of her step-father. The victim was examined at a nearby hospital where samples were collected for a Sexual Assault Evidence Collection Kit (SAECK). The victim stated that she had consensual vaginal sex ten hours prior to the sexual assault and that she wore body bronzer on her arms and legs.

The Waterbury Police Department submitted physical evidence from the victim's home (pieces of black electrical tape removed from the victim and a tube of body bronzer used by the victim), from the step-father's vehicle (mask and a pair of work gloves), from the step-father's home (roll of black electrical tape), the SAECK, and known samples from the victim's consensual sex partner (CSP) and the victim's step-father (suspect). The SAECK, mask and a pair of work gloves were examined and analyzed by the Forensic Biology Section. Samples from the SAECK, mask, right work glove and known samples from the victim, CSP and victim's step-father were forwarded to the DNA Section. Samples from the mask and right glove were forwarded to the Chemistry Section. Pieces of black electrical tape removed from the victim and the roll of black electrical tape obtained from the step-father's home were examined and analyzed by the Trace Section. Members from the Forensic Biology, Chemistry, Trace, and DNA sections of the CT Division of Scientific Services testified at a jury trial. The poster will reveal the associations made by the analysis of the physical evidence and the outcome of the trail.

Surface Modification Using 9,10-Diphenylanthracene For Fingerprint Enhancement

Elliot Quinteros, Karol Alvarez - John Jay College of Criminal Justice
Marcel A. Roberts,Ph.D. - John Jay College of Criminal Justice

Abstract:

Fingerprints are an important resource for identification purposes, especially for forensics applications. Commonly, latent fingerprints require enhancement by chemical or physical methods post deposition. Such methods depend upon the types of surfaces where the fingerprints are found. In order to change that paradigm, we propose to pre-functionalize various surfaces, which will help generate well-resolved fingerprints. 9,10-Diphenylantracene (DPA), an ultraviolet reactive dye, has that potential. DPA was utilized by creating a mixture capable of producing and generating well-define fingerprints for identification purposes. Such mixtures were obtained by diluting different concentration of DPA in water as well as in different organic

solvents such as water or methanol. Applying this mixture to different surfaces like clothing, glass, other polymers, corrugated paper, metal, wood and aluminum foil, allowed for the testing and determination of the most effective DPA mixture. Determinations were based upon observations of prints deposited on modified surfaces under white and ultraviolet light in which the ridge and minutia characteristics were readily observed. Based on data, 0.1% DPA in distilled water demonstrated better results producing a well define fingerprint. Using that combination, the surface with the best resolution was obtained on nonporous materials such as glass, polymer as well as porous materials such a corrugated paper. When compared to other commonly used methods such as black powder, cyanoacrylate and fingerprint ink, the ability of DPA to greatly enhance fingerprint resolution was significant. The ridge pattern and minutia characteristics were clearly present. For instance, the resolution of fingerprints obtained from a surface modified with 0.1% mixture of DPA is best compared with inked fingerprint on polymer surfaces as ridge patterns and minutia were more pronounced and easily recognizable for comparison. Results have shown that surface modification using DPA has not only the potential to generate well-resolved fingerprints for identification proposes, but it is easy to use, non toxic and, overall is relatively inexpensive.

Nothing is What it Seems: The Epidemic of Fake Pharmaceuticals

Nicole A. Bush - NMS Labs

Abstract:

This poster examines the role that counterfeit pharmaceuticals and herbal preparations are playing in our society and the appearance of counterfeits in the forensic laboratory. Counterfeits have been appearing in circulation at staggering rates, so it is no surprise that the influx of volume of these fraudulent supplies in the forensic laboratory is also on the rise. We are currently seeing many examples in our lab, from Oxycodone tablets made flawlessly to replicate commonly manufactured tablets but containing different active ingredients to tablets that contain compounds not listed in any general use GC/MS databases. From a global perspective, counterfeits pose a threat to society that we have yet to fully grasp. For big pharma, this threat is financial and to their reputation. For the public, this means serious health risks. But the problem will continue to rise as long as we have this situation where materials from overseas are cheap and easy to come by and the prosecution of drug trafficking for pharmaceuticals is so much leaner than that of pushing illicit street drugs. My poster will look at the problems this issue poses to the forensic drug chemist. These problems range from the indications that random sampling from within a population have on separating counterfeits from real preparations to performing a visual identification only on monogrammed non-controlled pharmaceuticals, presenting the opportunity to keep counterfeits circulating and doing a disservice to the public. This poster will present information regarding some examples of counterfeiting we see throughout our lab and will discuss information regarding the implications on a global level due to this epidemic.

Likelihood Ratios: Use and Issues in Forensic DNA

Heather Miller Coyle - Forensic Science Department, University of New Haven, 300 Boston Post Road, West Haven, CT 06516 USA, email: Hcoyle@newhaven.edu

Abstract:

A likelihood ratio (LR) is a statistical method for giving weight to a DNA match in testing. Likelihood ratios give a user one of two options: Hypothesis #1 (Hp, Prosecutor Theory) –that the individual in question is included in the DNA sample and Hypothesis #2 (Hd, Defense Theory) – that another unrelated individual is the most likely the source of the sample. Likelihood ratios have not been used often in DNA testing as historically report options include the following choices of included, excluded or inconclusive in most forensic DNA laboratories. Although statistically correct as a method, with likelihood ratios, there is an inherent error rate based on the fact that one must choose either inclusion or exclusion. Therefore, the category of samples that were formally reported as inconclusive are now by default classified as either inclusions or exclusions. This places those samples that were formerly reported as inconclusive with scientific accuracy into a potentially inaccurate category (false inclusion or false exclusion). In addition to use of LR as a reporting method, in complex mixtures, the ability to accurately source the correct DNA fragment to the correct individual is affected by many factors. These factors include stochastic effects, allele drop in rates or contamination, allele drop out rates, the correct assessment of number of contributors as well as percentage of shared alleles between contributors. All of these factors contribute in some way to the error rates or scientific accuracy of source attribution and reporting in DNA mixture interpretation. A variety of error rates from publically available information
will be presented.

Keywords: Likelihood ratio, false positive, source attribution, DNA, mixtures

Identification of Plant Evidentiary Material Using CBOL Guidelines

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3 National Marijuana Initiative, HIDTA-ONDCP Suite 1900, 1010 Second Avenue, San Diego, CA 92101.

Abstract:

Plant material is often found as associative evidence at crime scenes (leaves, grasses) or as illicit drug material (marijuana, “Spice” (synthetic marijuana) products). Identification of these materials is not always straight forward due to fragmentation, shared morphological characteristics, lack of chemistry or designer cannabinoids spiked on other plant species. In the case of “Spice”, this refers to dried shredded plant materials that are purported to have natural psychoactive effects due to addition of cannabinoids to the surface. Surveys of high school students show that use of “Spice” is second only to authentic marijuana and the identity of the substance can be difficult as the cannabinoid chemistry is constantly changing. In these cases, DNA sequencing of the plant material can be used to make a simple conclusive identification or distinction

between synthetic and authentic. DNA can be a useful tool for looking at provenance, percentage use of base materials and potential toxicology (abuse and overdose) from interactions of chemistry and plant substances. The Consortium for the Barcode of Life guidelines were used for locus selection (www.barcoding.si.edu/protocols.html) which concludes that rbcL or matK loci independently or combined provides a universal framework for barcoding DNA of land plants. A public access DNA database of approximately 82,853,685 vouchered sequences is available as a reference library for making comparisons (a plant CODIS system) from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). As a test case from a scene, a seed sample from a cover crop collected from a marijuana grow site was sent to the UNH DNA Laboratory for identification. The seed was difficult to identify by microscopy as many grass samples have similar appearance and fungicide obscured the surface details.

After DNA extraction, PCR amplification of the rbcL locus and TOPO capture cloning of PCR product, automated DNA sequencing yielded an identification of the sample as barley (*H. vulgare*, AY137456). Additional sequencing of marijuana samples has confirmed use of rbcL for identification. This methodology can yield added information for trace plant evidence from crime scene or drug sample and is analogous to test methods used for hair identification (microscopy followed by DNA) in forensic science. Data mining for relevant reference barcodes to create a “plant CODIS” of DNA sequences is in progress.

Keywords: barcode of life, DNA sequences, rbcL, species identification, plant CODIS

***The Deposition of Gunshot Residue in Conditions of Rain and Fog**

Stacey Ishmail - John Jay College of Criminal Justice
Mentor/Research advisor - Peter Diaczuk, John Jay College of Criminal Justice

Abstract:

This project explores how gunshot residue is deposited onto a target in ambient air, rain, and fog. An objective method is being explored for examining the density pattern of gunshot residue by using the computer software ImageJ. The advantage of this method is that it is inexpensive and does not involve the use of chemicals, which can alter the state of the particles. Due to their different aerodynamic capabilities, the propellant types used in this project are flattened ball and disc shaped. These were loaded in Winchester 38 Special and Federal American Eagle 38 Special cartridges, respectively. The muzzle-to-target distances for each shots are 8 and 12 inches. In the ambient air conditions (with no precipitation), it was shown that the type of ammunition, propellant particles, and shooting distance determines the gunshot residue pattern deposited on to a target. The rain and fog conditions were created artificially in order to conduct this experiment within the controlled setting of the laboratory. A jig was designed to deliver water on demand in reproducible known volumes, measured in gallons per minute. The water droplets so created became an intervening medium that the unburned and partially burned propellant particles had to negotiate on their way to their target. The method for examining density pattern in ImageJ can be crucial in the reconstruction of shooting incidents that occurred under conditions of rain and fog.

*** Collegiate Competition**

Northeastern Association of Forensic Scientists

***Characterization of the sources of peak height uncertainty resulting from ordinary alterations during forensic DNA processing: Examining validation schemes for the calibration of NOCIt**

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Abstract:

Forensic DNA mixtures may be complex and comprised of any number of contributors in any proportion. In these cases, classical DNA interpretation schemes, such as CPE/CPI, may not allow for comparison between standards and evidence. In response to the issues associated with complex, low-template analysis, several methods and/or recommendations to determine the likelihood ratio, which requires an assumption regarding the number of contributors (NOC), have been published. Classical approaches to determining the NOC have relied on counting methods. However, as a DNA profile becomes more complex, the minimum NOC may not be equal to the actual number. To accurately assess the probability that a certain NOC gave rise to an evidentiary item, the probability of drop-out (Pr(DO)), baseline noise, and stutter proportion, previously characterized via validation, must be considered. Since proper interpretation relies heavily on validation and calibration results, it is necessary to identify and characterize any sources of peak height uncertainty that results from natural changes of laboratory factors. It is also necessary to confirm that these alterations do not significantly impact the thresholds or probabilities, which are then used to establish the results obtained from NOCIt.

This study focuses on the characterization of uncertainty associated with injection, capillary lot, amplification, kit lot and sample. Three samples were amplified at six targets and run in quadruplicate. To test injection variation, one sample preparation was injected four times on one capillary.

Another sample preparation was injected 4 times, changing the capillary lot before each injection. The amplification variation was measured by amplifying the samples in quadruplicate using one kit lot. Amplifying 3 additional times with 3 different kit lots was done to measure kit lot variation. The peak height results were summarized by examining height concordance for every allele at different target amounts. The data was separated by target, the parameter being tested, sample number, locus, and allele. Each of the observed peak heights for a given allele was then divided by the largest observed peak height for that allele (PH_{max}), resulting in a ratio between 0 and 1. For parameters with high levels of reproducibility, all four peak height ratios will be close to 1.

Data indicate that the uncertainty associated with the amplification has the greatest effect on the reproducibility of the RFU signal, followed by kit lot then capillary then injection. For example, the signal originating from a 0.25 ng target resulted in concordance ratios of 0.97 ± 0.02, 0.89 ± 0.07, 0.72 ± 0.15 and 0.71 ± 0.16 for injection, capillary, amplification and kit lot changes respectively. Similar results were observed for all 6 targets considered. Changes in baseline noise were also examined and preliminary results show that baseline signal was not significantly impacted by kit lot, amplification, capillary lot, or injection.

Therefore, when calibrating NOCIt or when determining stochastic thresholds and/or rates of drop-out, the validation dataset should include, at the least, amplifications of multiple different sample dilutions series, run such that multiple capillary lots are included during calibration.

*** Collegiate Competition**

***Optimizing the Extraction of Gamma Hydroxybutyric Acid from Hair for GC/MS Analysis**

Kaila Kleckner and Lawrence Quarino - Cedar Crest College, Allentown, PA

Abstract:

Gamma hydroxybutyric acid (GHB) is a short-chain fatty acid that was designed for medicinal purposes but has become a recreational drug of abuse. Due to its sedative and amnesia effects, GHB is becoming increasingly seen in drug-facilitated sexual assault cases. While GHB can be detected through urinalysis, its half-life of 20-53 minutes makes detection difficult after twelve hours. Since many sexual assaults are not reported within that twelve-hour window, hair has become a common matrix for GHB detection. Many common hair analysis procedures are time consuming, and require a significant amount of sample preparation. The use of freeze/thaw cycles lessens not only the time consumed, but also the amount of preparation required. Hair is cut and weighed to approximately 40 mg, then placed in 1 mL of extraction solvent; ethanol and ethyl acetate were tested in this study. The samples are then frozen and allowed to thaw in the extraction solvent, which is then dried, derivatized with BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) + TMCS (trimethylchlorosilane) (99:1), and reconstituted in acetonitrile. Analysis is performed using a pulse-splitless GC/MS method and an initial oven temperature of 60°C with four ramps to a final temperature of 310°C. The overall run time for the method is approximately 14.70 min. Calibration curve shows linearity from 0.0001 mg/L, with a visual limit of detection determined at 0.0001 mg/L. The current freeze/thaw method indicates the use of ethanol as a better extraction solvent and that sonication is important for yielding consistent results. Test runs using ethyl acetate yielded no results. Levels of endogenous GHB extracted from hair ranged between 0.101 ng/mg and 5.65 ng/mg which is consistent with reported values.

*** Collegiate Competition**

Best Practices for the Use of Micropipettes

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Abstract:

Mechanical action micropipettes are ubiquitous in laboratories and are used for many routine tasks, including the quantitative measurement and dispensing of analytical samples and reagents. Concentrations of biological and chemical components in the prepared samples for assays and tests are volume-dependent and incorrectly performed pipetting steps will directly impact the transferred volumes, and hence, the test results. The design and construction of these pipettes render their performance susceptible to the technique and skills used by the operator of such devices. This poster describes the basic principles related to an operator's pipetting technique, and quantifies the errors induced by using improper techniques. For example, not pre-wetting the pipette tip may induce up to 1.5% error in the dispensed volume, which may be added to another 1.5% variation induced by the length of the pause before removing the pipette tip from the sample solution. Adding to the cumulative error in pipetting are inconsistencies in aspiration and dispense speeds and applied pressure on the plunger (up to 0.5%), heat transfer from the hands (up to 1.8%), improper immersion depth of the pipette tip into the sample (up to 1.3%), and the choice of the pipetting mode (up to 2.5%). For obtaining reliable laboratory test results it is imperative that all pipette operators are consistently using the proper pipetting technique. Training pipette operators on using the correct pipetting technique ensures confidence in the test results on which a clinical diagnosis is based, and is equally critical in assay transfer situations or whenever results from different operators and laboratories need to be compared to each other.

A Survey of Techniques and Their Applicability to the Analysis of Emerging Synthetic Cathinone Derivatives

Mentor: Jeannie Guglielmo
Jen Goshaw; Songkai Hu; Kareem Ibrahim; Maelissa Lim; Adam Sepe

Abstract:

In 2010, when poison centers across the United States first began to monitor the use of synthetic cathinones (known colloquially as bath salts,) only 302 calls indicated that these drugs were involved. As of October 3, 2011 this number had jumped to 5,226 (Olives et al 59). These statistics may only be a small fraction of what is otherwise undiagnosed or unreported bath salt abuse. Non-synthetic cathinone is a naturally occurring psychotropic drug that can be found in the leaves of the *Catha edulis* plant. This plant has been chewed for many years by the people of East Africa and the Arabian Peninsula (Brenneisen et al 1). Synthetic cathinones have been manufactured for almost a century but did not gain much recognition in more developed countries until recently. According to one study, there are currently 37 separate "brands" distributed under many monikers (*Bath Salts, Ivory Wave, Vanilla Sky*, and others) in the form of cathinone derivatives: mephedrone, MDPV, etc. Primary clinical effects are neurological and cardiovascular and can include: agitation, tachycardia, paranoia, chest pain, mydriasis and blurred vision (Spiller et al. 499). These substances also have behavioral and physiological effects which are similar to those of methamphetamine and ecstasy. Furthermore, synthetic cathinone abuse can be just as life-threatening as these more well-known drugs (Olives et al 58-60). Unsurprisingly, the recent trend in the illicit use of synthetic cathinones within the past two years has gained not only the attention of local emergency and police departments, but that of state and federal government as well. Contrary to popular belief, and due to more stringent legal action, the acquisition and purchase of

"bath salts" has become more difficult. These substances, previously sold at tobacco and head shops have disappeared from many stores throughout the United States. Recent Suffolk County, NY legislature (Nov. 2011) details the ban of specific synthetic cathinones including "Methylone", "Mephedrone", and "MDPV."

Synthetic cathinones, which include 3,4-methylenedioxypyrovalerone (MDPV) and mephedrone, are psychotropic drugs that can cause hallucinations and various other severe conditions. In recent years they have become a very popular class of drugs due in part to the lack of strict regulations on their possession and use. The purpose of this review is to investigate the applicability of current methods for the analysis and detection of synthetic cathinones. These techniques included: gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), screening assays like thin-layer chromatography (TLC), and newer advanced technologies. Through literary research the strengths, weaknesses, and current status of each technique was determined. Comparisons of multiple screening kits were made during the survey. The major noteworthy differences were seen in the areas of cost, sensitivity, specificity, timeliness and portability. Further studies can involve the application of upcoming methods to clinical specimens such as blood and urine, creating reference material and standards libraries, developing new methodologies, and continuous monitoring of emerging technologies and trends. References: Contact Authors

Development of an Immuno-Magnetic Procedure for the Separation of Spermatozoa from Vaginal Epithelial Cells

Margaret M. Wallace, PhD, Christine Bless. MS & Lillian Guia, MS - John Jay College of Criminal Justice

Abstract:

The main goal of this NIJ grant (2011-NE-BX-K546) funded research is to develop a simple, robust, cost and time efficient method for the separation of spermatozoa from vaginal epithelial cells. Seven mouse antibodies directed against human sperm surface antigens located in the head region were chosen for this study: polyclonal anti-AKAP3, polyclonal anti-SPAM1/PH-20, monoclonal & polyclonal anti-fertilinβ/ADAM2, monoclonal and polyclonal SED1/ MFGE8, and polyclonal anti-UBAP2L. Each antibody was evaluated for its ability to capture human spermatozoa in single source samples and to produce quality DNA profiles.

Varying numbers –20,000, 2,000, 200, 20, and 2– of sperm cells were added to the 5 ug of each antibody in separate 0.5 ml sterile microcentrifuge tubes. PBS buffer was used as the negative control for each antibody set. Vaginal epithelial cells –400– were used to test cell type specificity.

Twenty-five µl of Protein G were used for the formation of the immuno-magnetic complexes. The "captured" cells from the samples and controls as well as the "wash solutions" were stored at 4 ºC.

DNA from the immuno-magnetic complexes and "wash solutions" was extracted using the Chelex method, concentrated using Amicon Ultra centrifugal filters, quantified using the Quantifiler Duo® DNA Quantification kit and typed using the AmpFlSTR® Identifiler Kit. DNA from buccal swab samples was extracted, quantitated and typed for comparison.

Preliminary typing results indicated that four of the antibodies- polyclonal anti- AKAP3, monoclonal SED1/ MFGE8, monoclonal anti-fertilinβ/ADAM2, and polyclonal anti-UBAP2L successfully captured sperm cells and produced quality DNA profiles. Studies using varying amounts of antibody to capture single samples are in progress to evaluate the efficacy of these antibodies.

Towards an expanded detection of new synthetic cannabinoids by means of immunoassays

Eyer A, McCosh L, Taggart, J, Frew J., Vintila I, Savage S, Darragh J, Benchikh ME, McConnell RI, FitzGerald SP - Randox Toxicology Limited, 30 Cherryvalley Road, Crumlin, United Kingdom, BT29 4QN

Abstract:

Synthetic cannabinoids are chemical compounds that mimic the effects of tetrahydrocannabinol, the main active ingredient of cannabis. Originally sold under the brand name “Spice”, this brand name has become a generic term to include the entire class of “legal” smoking blends sold on the internet. The generation of new synthetic cannabinoids is continuously evolving, to circumvent legal restrictions. The two most common first generation synthetic cannabinoids are JWH-018 and JWH-073. One cannabimimetic recently identified is AB-001, urinary metabolites have been reported (the major metabolites were found to be adamantane mono hydroxylated and adamantane mono-hydroxylated/N-dealkylated products).The parent compound was reported to be absent in human urine sample. RCS-8, is a synthetic cannabinoid described as an analogue of JWH-250. Despite not having been reported in the scientific or patent literature as yet, reputed recreational use of RCS-8 in the United States has led to it being specifically listed in a proposed 2011 amendment to the Controlled Substances Act, which is aiming to add a number of synthetic drugs into Schedule I. UR-144 (a synthetic derivative of JWH018) and its fluorinated version XLR-11 are the new generation of synthetic cannabinoids. These new compounds are potent and addictive, and account for up to 80% of confirmed positive findings in synthetic cannabinoid-containing blood samples. The aim of this study was to develop antibodies against recently identified synthetic cannabinoids for the development of new immunoassays to expand the detection of these compounds, which is relevant in drug testing settings.

Methods. The immunogens comprising AB-001 hapten conjugated to bovine thyroglobulin (BTG) and RCS-8 hapten conjugated to BTG and UR-144 hapten conjugated to BTG as a carrier protein were separately administered to adult sheep on a monthly basis to provide target-specific polyclonal antisera. IgG was extracted from the antiserum and evaluated via competitive immunoassay (ELISA).

Results. AB-001 polyclonal antibody: the assay was standardised to the AB-001 N-(pentanoic acid), AB-001 N-(5-hydroxypentyl) (%cross-reactivity: 40%) was also detected, and the %cross-reactivity with the parent compound was of 7%. The % cross-reactivity with JWH-018, JWH-073 and JWH-250 was < 3.7% and with UR-144 N-(pentanoic acid), UR-144 N-(5-hydroxypentyl) and XLR-11 was >10%. The sensitivity, expressed as half maximal inhibitory concentration (IC_{50}) was 0.744ng/ml. RSC8/JWH-250 polyclonal antibody: the antibody presented %cross-reactivity 100% with JWH-250 and also showed >25% cross-reactivity with RCS-8. The IC50 value was <0.5ng/ml. UR-144/XLR-11 polyclonal antibody: The assay was standardised to UR-144 N (pentanoic acid) and showed 19% cross-reactivity to the UR-144 parent molecule. A broad range of additional UR-144 compounds were detected: UR-144 N- (4-Hydroxypentyl Metabolite (107%), UR-144 N-(5-hydroxypentyl) metabolite (110%), and UR-144 N-(Desalkyl) (13%). The assay also detected XLR-11 at 29% and A-834735 at 111%. The IC50 value was 0.702ng/ml.

Conclusion: This initial evaluation shows the development of polyclonal antibodies for the detection of recently identified synthetic cannabinoids. The results indicate that these antibodies are suitable for the development of immunoassays for the generic determination of these compounds. This is relevant for the development of new immunoassays to extend the screening of synthetic cannabinoids in drug testing settings.

Accuracy Matters When Quantitative, Manually-pipetted PCR Assays Transfer to Automation – A Story in Diagnosing and Troubleshooting

Keith J. Albert - Artel, Inc., 25 Bradley Drive, Westbrook, Maine 04092
Lisa M. Knapp - Agilent Automation Solutions, 5301 Stevens Creek Boulevard, Santa Clara, CA 95051

Abstract :

It is often the case that assays are initially performed on the benchtop using handheld pipettes before they are transferred to an automated liquid handler. Automating a manual method may take time and patience, but automation will help lower costs, increase throughput, and potentially avoid errors associated with a manual method. During the transfer process, however, the manual assay should be directly compared to the automated assay for consistencies in pipetting performance. An undetected variability in accuracy will impact the integrity of the assay as the automation process continues. Liquid handling accuracy and precision information, for both the manual and automated method, are critical to determine any deviation of dispensed volumes between the two processes. Therefore, as it is shown in this presentation, that validating the liquid delivery steps for each assay will help uncover discrepancies in pipetting performance. This presentation discusses the importance of knowing both accuracy and precision information when a manual method is transferred to a robotic liquid handler. It was determined that the rate-limiting reagent in the RT-PCR assay was not being accurately pipetted between the manual and automated methods per the protocol, and the automation was not to blame.

Screening analysis of Synthetic Cannabinoids by using a HPLC – UV – Fl method

E. Trapani[1], A. Vandoros[2], E.F. De Palo[1],T. Palmbach[2], F. Tagliaro[1]
[1] Department of Public Health and Community Medicine, Unit of Forensic Medicine, University of Verona, Verona.
[2] Forensic Science Department, University of New Haven, West Haven, CT, USA.

Abstract:

Synthetic Cannabinoids (SC) represent a very important side of the emerging and worrying phenomenon of the New Psychoactive Substances (NPS).

Currently, the determination of synthetic cannabinoids is based on the use of LC/GC - Mass Spectrometry techniques . Although this analytical approach have been successfully applied to the determination of SD in both clandestine preparations and biological fluids [1], it is expensive and usually not suitable for screening analysis in routine laboratories.

Aim of this work was the development and the validation of a method based on a HPLC separation associated with a UV and fluorimetric detection for the determination of SC in herbal mixtures.

The experiments were performed using a HPLC-UV-FL system [LC-10AD HPLC Pump (Shimadzu, Tokio); Jasco 875-UV Intelligent Spectrophotometric detector (Japan); RF10XAL Fluorescence detector (Shimadzu)]. A reversed phase C18 column [Discovery, 150 mm x 4.6 mm, 3 µm, (Supelco, PA, USA)] was employed. The Communication Bus Module was a CBM-20A Prominence (Shimadzu) and the program on PC was "LabSolution" (2008-2010, Shimadzu Corporation). The separation was isocratic with a mobile phase composed by MetOH/H_2O (87,20/12,80, v/v); the flow was set at 1 ml/min; the pressure was ≈ 200 bar. After optimization the best UV absorption was at 229 nm, while, for the majority of the compounds, the best fluorescence signal was obtained at excitation wavelength of 350 nm and emission wavelength at 425 nm.

The sample preparation of herbal mixtures was based on a simple extraction procedure (≈20 mg of sample incubated o.n. in 2 ml of methanol at room temperature).

Under the described conditions, nine synthetic cannabinoids were separated within 10 minutes with the following elution sequence: JWH-200, AM-694, JWH015, /-250, /-073, /-018, /-081, /-019, /-210. Linearity was tested in the 1-10 µM concentration range. The analytical sensitivity ranged from of 5.9 ng/ml (JWH-081) to of 60 ng/ml (JWH-073).

The method was successfully applied to the analysis of herbal mixtures seized at Smart Shops.

References - [1] Meyer MR, Peters FT. Analytical toxicology of emerging drugs of abuse—an update. Ther Drug Monit. 2012; 34:615-21.

Optimization and Validation of the EZ1® DNA Tissue Kit for the Purification of DNA from Sexual Assault Samples

Lana Ramos, M.S., Carll Ladd, Ph.D., John Schienman, Ph.D., Angela Przech, Ph.D.
State of Connecticut Department of Emergency Services and Public Protection Division of Scientific Services 278 Colony Street, Meriden CT 06451

Abstract:

The Connecticut Department of Emergency Services and Public Protection Division of Scientific Services recently optimized and validated a protocol for the purification of DNA from sexual assault samples using the EZ1® DNA Tissue Kit with the EZ1® Advanced XL. Compared to current manual organic extraction procedures, this automated method recovers an adequate amount of clean genomic DNA from samples, while saving analyst's time.

The QIAGEN® recommended protocol for the pretreatment and purification of epithelial cells mixed with sperm cells using the EZ1® Advanced XL was optimized in order to maximize DNA recovery. Maximum DNA recovery may be critical in being able to obtain a Y-STR profile when the overwhelming majority of the sample is female DNA. Variables tested that significantly affected the amount of DNA recovered include digestion buffer (In-house buffer[1] vs. QIAGEN® Buffer G2[2]), and reagent kit (EZ1® DNA Investigator® Kit vs. EZ1® DNA Tissue Kit).

Internal validation of the EZ1® Advanced XL included studies on reproducibility, sensitivity, known and mock samples, manual comparison, and cross-contamination.

[1] In-house buffer contains: Tris-HCl, EDTA, NaCl, SDS
[2] QIAGEN® Buffer G2 contains: Guanidine-HCl, EDTA, Tween-20, Triton X-100

Modification Of An Existing Robotic Differential Extraction Procedure

Kristin Alfano - University of New Haven; Joseph Galdi - Suffolk County Crime Lab

Abstract:

The Suffolk County Crime Lab currently utilizes a differential DNA extraction procedure combining the Promega Differex kit with the Qiagen EZ1 DNA Investigator Extraction Kit. The procedure includes a two hour incubation time prior to placing the samples in the Biorobot EZ1 Workstation. The purpose of this project was to determine if the incubation time could be shortened. Duplicate samples were extracted using incubation times of 2 hours, 1.5 hours, 1 hour, 30 minutes and 15 minutes. All of the DNA extracts in this study produced equivalent yields of DNA. In addition, all samples gave full DNA profiles. Therefore, the 15 minute incubation time may be used, significantly cutting down on the time of the procedure.

Synthetic Cannabinoids and Designer Drugs in Connecticut; 2011 – 2013

Korey Dabrowski, Laura Grestini and Robert H. Powers, Ph.D., DABFT; University of New Haven, West Haven CT, and CT DESPP Controlled Substances/Toxicology Laboratory, 278 Colony Street, Meriden CT 06451

The pattern of synthetic cannabinoids and designer drugs seized in Connecticut, and throughout the nation has changed during the last several years, and new species continue to appear in the analytical workflow. We have tabulated specific compounds identified from 2011 – 2013, and discuss the pattern of seized drugs during the period, both in terms of the number of cases, and the frequency with which specific compounds within drug groups were seen, as well as the scheduling classification. Amongst the cannabinoids, the most prevalent species in 2011 was JWH-18, supplanted in 2012 by AM2201, So far in 2013 XLR-11 is the most prevalent. Evolving structural variations within the cannabinoids, and other drug groups are discussed. Specific compounds detected in CT for SMA's cathinones, piperazines, and tryptamines, and numbers of cases are also presented, as well as unique or unusual analogue species. Implications with regard to analogue designation, and the criteria for such classification of specific compounds are considered as well.

Reunification of an Ashkenazi Jewish Family Separated by the Holocaust Using Y-Chromosome Lineage Analysis.

Lindsay Schulman, Jamie L. Wilson, Sam Silverman, and Bruce A. Jackson - Forensic DNA Science Program, Department of Biotechnology, MassBay Community College, Wellesley Hills, MA; + Olaf Pharmaceuticals, Worcester, MA

Abstract

Y-chromosome lineage analysis is very useful for confirming a relationship between relatives separated by various circumstances. In this study, we attempt to reconnect five Ashkenazi Jewish men from three different continents who share an oral history of a male ancestor with the surname K/Corenbaum from the Bug River region in modern-day Belarus. In each oral history was a strong reference to familial separation during the Holocaust. We used four Y-SNPs and 12 Y-STR markers to determine the haplogroup and exact haplotypes for the five Jewish men. aWe determined that all five individuals possessed the same haplotype and belonged to the Near East haplogroup E1b1b1c. This analysis confirmed that all five individuals shared a common paternal ancestor. However, the number of generations removed from that common paternal ancestor could not be determined. We conclude that Y-chromosome lineage analysis is a powerful tool in lineage confirmations of displaced peoples. However, its usefulness is greatly diminished if not used in conjunction with other investigative tools. We describe a study of the Korenbaum family as a model for the reunification of displaced families using Y-DNA analysis.

Northeastern Association of Forensic Scientists

Drug Chemistry Abstracts

***Screening of Confiscated Synthetic Cannabinoid Samples**

Michael O'Donnell, Philip Burlingame and Monica Joshi, PhD - 750 South Church St. Department of Chemistry, West Chester University of Pennsylvania, West Chester PA 19383

Abstract:

The recent influx of synthetic cannabinoid products has created analytical and legal challenges in the forensic community. The evolving structures and increasing complexity of the products has devalued the use of simple screening techniques and canine detection. The discussed research is a collaborative effort between West Chester University of Pennsylvania and the Pennsylvania Department of Corrections (PA DOC). The purpose of the study is to analyze the herbal materials that are confiscated by the PA DOC in their correctional facilities for the presence of synthetic cannabinoids. The results of these studies will be used to assist the Department in assessing emerging trends and in facing the increasing challenge of herbal materials at their facilities. The overall research goal is to develop a database of synthetic cannabinoids and associated psychoactive substances that could be used for developing novel detection methods.

Attendees at this presentation will be introduced to non-destructive sample preparation techniques and their effectiveness in extracting synthetic cannabinoids from confiscated herbal materials. The study describes the use of gas chromatography- mass spectrometry (GC-MS) as the analytical technique coupled with different sample preparation techniques. The extraction of cannabinoids from the herbal material using solid phase microextraction (SPME) and liquid phase microextraction (LPME) is compared to traditional liquid extractions reported in literature. Samples that were previously tested by GC-MS techniques by another laboratory were also analyzed by our method. The presentation will discuss the results of headspace analysis of the products and the complications associated with the unknown herbal materials. A complete chemical profile of the herbal materials requires a combination of extraction techniques.

***Collegiate Competition**

Evaluation of the TruNarc Handheld Raman Device as a Tool in the Examination of Controlled Substances

Sandra Meier, Elizabeth Marks - Suffolk County Crime Laboratory

Abstract:

Raman spectroscopy has been considered a discriminating method of analysis for nearly 80 years. It has been classified as a SWGDRUG Category A technique, due to its discriminatory powers. Despite the fact that it has been deemed a suitable method of molecular confirmation, Raman spectroscopy is not readily used or available in many forensic drug laboratories.

The Suffolk County Crime Laboratory routinely issues reports on preliminary examination of tablets. The analysis consists mainly of a literature search based on the appearance and markings of those tablets.

Recently, the laboratory has experienced increasing requests from the courts for more in-depth preliminary analysis, which for tablet submission, would often require extractions and thin layer chromatography, thereby increasing analysis time with no corresponding increase in manpower. At the last NEAFS meeting, a presentation was given on the TruNarc and its ability to perform Raman spectroscopy on samples with little or no sample preparation. This was viewed as a possible solution to the laboratory's current situation. The laboratory recently acquired a TruNarc handheld Raman spectrometer in an effort to meet the demands of the requesting agencies.

Upon receiving the instrument, the first step involved verifying that the instrument could correctly identify known standards. Controlled and non-controlled substances were tested and results were recorded. For inconclusive and/or inconsistent results, TruNarc customer support was used. TruNarc customer support provides a "reach back" function whereby spectral data can be more closely examined and interpreted to provide assistance in analysis. The next step was to develop a procedure detailing the types of cases to be examined with the device as well as steps to take when encountering an "inconclusive" result.

This talk will discuss the basics of Raman spectroscopy, the functionality of the instrument, sample preparation, availability of customer support, advantages and limitations of the instrument, how the Suffolk County Crime Laboratory applies the instrument to casework and issues that were encountered during the process.

Validation of Microscopic Marijuana Analysis

Jim Wesley, Maria Soures - Monroe County Crime Lab, Rochester, NY

Abstract:

Scope: Validation is an important part of method documentation. Because Marijuana has specific recommendations for testing (SWGDRUG Recommendations 2011, 3.2.1) it is important that chemists have confidence that their microscopic examinations are supported by a validated method. Regarding Marijuana, we must have documentation that commonly available substances; cooking spices and medicinal/psychoactive herbs in particular, have microscopic characteristics that are dissimilar from those found in marijuana.

Method: We used photographic techniques to produce reviewable documentation (digital photographs) to substantiate the microscopic examination of Marijuana. A Canon camera system using a 7D camera, F 2.8, 100 mm lens and the very cool MP E-65 microscopic lens was used. The plant materials were also photographed using an Infinity 2 (Lumenera) video camera mounted on an Olympus SZ61 stereomicroscope. This system is capable of 6.7-45x magnification.

Dried plant substances (Cooking Spices and Medicinal/Psychoactive Herbs) were examined using the same techniques that we apply to the examination of Marijuana. A gallery of microscopic photographs was produced and will be circulated. The gallery supports the validation and enhances training. Analysis of the substances by Duquenois Levine and GC/MS will also be detailed.

Canon 7D Lens Settings		
F 4-5.6, 17-58 mm Zoom Lens		
47 cm	~ 6 inches (Min)	
47 cm	~ 18 inches (Max)	
F 2.8, 100 mm Macro Lens		
Copy Stand Height	FOV	Microscope Setting
47 cm	58 mm	
44.5 cm	52 mm	
38 cm	36 mm	
35 cm	26 mm	
33 cm	22 mm	10 X (22 mm FOV)
MP E-65 Microscopic Lens		
Copy Stand Height	FOV	Microscope Setting
27 cm	22 mm	10 X (22 mm FOV)
26.5 cm	11 mm	20 X (11 mm FOV)
28.5 cm	7 mm	
31 cm	5 mm	40 X (5 mm FOV)
33.5 mm	4 mm	

Analysis of Marijuana Street Samples for Simultaneous Potency and Pesticide Fingerprinting Using a Deans Switchwith GC-FID and GCxGC-ECD

Lindsay Mitchell[1], Emily Ly[1], Amanda Leffler[1], Jack Cochran[1,2], Julie Kowalski[2] and Frank L. Dorman[1]
[1]The Pennsylvania University, University Park, PA 16802
[2]Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

Abstract:

Law enforcement already integrates the use of GC-FID to test for cannabinoid potency in marijuana samples. Given the legalization of medical marijuana, a need to better analyze marijuana samples is highly desired. While potency is monitored to determine strength of medical samples, it is thought that fingerprinting pesticides present in each sample could also be highly important. Experience has shown that most cannabis is treated with various pesticides, fungicides, and insecticides depending on the grower. The presence of these organic pesticides could present a safety issue, as they would not be ideal to have in medical marijuana samples. Additionally, the use of certain pesticides varies widely between samples, so pinpointing exactly which pesticides are in each sample could help identify a specific source for the sample and aid in the determination of trafficking patterns.

Cannabinoids and organic pesticides appear in marijuana sample chromatograms at very different ratios/concentrations. Given the large size of the cannabinoid peaks compared to the pesticides, it can be hard to simultaneously measure the pesticides with a standard chromatographic analysis. It is thought that using a Deans Switch would help “cut” out the larger cannabinoid peaks so that a GCxGC pesticide fingerprint could be determined for each sample, simultaneous to the cannabinoid profile.

This method integrates a Deans Switch into the GCxGC-FID/ECD, which allow for the GC-FID analysis of the cannabinoids for potency while the GCxGC-ECD provides the pesticide fingerprint in one method. Also, investigations into using a TOFMS system, in addition to the FID/ECD approach will be discussed to compare and contrast the two approaches. Optimizing one method that could determine potency and fingerprint trace pesticides could be instrumental for law enforcement to determine sources for various street and medical marijuana samples with a single method that may reduce costs and time.

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Direct Analysis in Real Time Mass Spectrometry (DART-MS) of Cathinone "Bath Salt" Drugs and Mixtures

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[3]JEOL USA, Inc., 11 Dearborn Rd, Peabody, MA 01960, U.S.A.

Abstract:

Synthetic cathinones, also known as bath salts, are a class of designer drugs that are marketed as alternatives to illegal psychoactive compounds like methamphetamine and ecstasy. Cathinones are highly addictive and their use has been linked to multiple overdoses and deaths. Although recent legislation in the U.S. has attempted to restrict these compounds, the vagaries of analog laws means that only a few specific compounds have been legislated while others have indeterminate legal status. With new derivatives of cathinones continually surfacing, controlling these substances is challenging.

GC-MS is a common method for detecting cathinones, however cathinones are found in a salt form so generally require extensive sample treatment before analysis. For example, GC/MS analyses require that samples are pH adjusted, extracted through liquid-liquid extraction, and derivatized. Furthermore, cathinones also degrade over time and often give very weak parent peaks, if present at all, making it particularly difficult to distinguish between isobaric compounds. Taken together, these factors contribute to the large number of backlogs in crime labs.

Ambient MS methods with high-resolution mass accuracy increase throughput of analyses as well as reveal more detailed structural information. DART-MS is one such ambient technique where solid material, such as an unknown white powder, can be ionized directly without extraction or chromatography, greatly reducing analysis time. With a time-of-flight mass spectrometer having a mass accuracy tolerance of five milli-mass units, more detailed structural analysis is possible. As a preliminary screening tool, DART-TOF-MS has the potential to reduce backlogs and streamline analysis of unknowns.

Here, DART-MS is used to identify and characterize individual cathinones, cathinone mixtures, and cathinones with various cutting agents. Low voltage spectra can provide parent $[M+H]^+$ peaks indicative of the number of compounds present, with high mass accuracy key in predicting formula weights. Multiple cathinones, including diethylcathinone, methylethcathinone, and fluoromethcathinone, were simultaneously fragmented by in-source collision induced dissociation (CID) to produce fragment patterns for structural characterization. In-source CID was also used to identify and differentiate isobaric cathinones, including diethylcathinone and 2-ethylcathinone, based on characteristic fragments and their intensities. Furthermore, a mixture of the two cathinones shows that individual components can be distinguished in a mixture of multiple drugs. DART-MS was also used in distinguishing drugs within cutting agents, including caffeine and lidocaine. High mass accuracy allows for rapid identification of common cutting agents, providing a means to analyze drugs in more complex mixtures. With no sample preparation needed, spectra were obtained in seconds, and the DART-MS

CID capability allows for both high mass accuracy molecular weight identification and fragmentation for structural characterization of unknowns.

After this presentation, attendees will gain an understanding of direct analysis in real time mass spectrometry (DART-MS) and its use as a rapid screening tool for drug analysis. The research presented here showcases a novel method for analysis, and discusses recent efforts to keep pace with the rapidly changing field of synthetic street drugs.

Analysis of Phenethylamine Street Drugs for Psychoactive Compounds and Impurities

Maura McGonigal, Philip B. Smith, Ph. D. - The Pennsylvania State University, 323 Life Sciences Building, University Park, PA 16802 and Frank L. Dorman Ph. D. - The Pennsylvania State University, 107 Whitmore Laboratories, University Park, PA 16802.

Abstract:

The purpose of this work is to determine not only the identity of the psychoactive compound/s, and their concentrations in the various street samples, but also to determine impurities which may exist from less than ideal synthetic procedures likely employed by potential users/manufacturers of “2C-type” drugs. Serious health complications and fatal overdoses have brought phenethylamine designer drug use to the public’s attention. The phenethylamine compounds alone are not believed to be causing the health complications, but rather the cause may be impurities with in the sample. These impurities may result from the improper technique and inadequate equipment used during illegal synthesis. The substituents on these emerging drugs are constantly changed in order to avoid legal ramifications. However, many of these compounds are Schedule 1 drugs, and therefore, their analogs are also illegal according the Federal Analog Act. These compounds are 2C-X series analogs of mescaline. The name “2C” results from the two carbons in the ethyl chain. These synthetic drugs are marketed as having affects similar to LSD and MDMA and are typically consumed sublingually via blotter paper. The compounds have psychedelic affects on the $5HT_{2A}$ receptor in the brain. The compounds have a variety of street names including “N-BOMB,” “Smiles,” and “Bromo-DragonFLY.”

The objective of this research is to qualitatively and quantitatively identify the drugs and potential impurities. Street samples will be compared to known standards in order to determine if impurities exist that may be resulting in health complications. The analysis will be done using a variety of ultra-high performance liquid chromatography (UHPLC) instruments. Liquid chromatography will be utilized rather than gas chromatography because the 2C compounds are considerably more reactive than many other recreational drugs, and analysis by gas chromatography has proven troublesome, at best.

Additionally using gas chromatography would require derivatization, which generally utilizes aprotic solvents, which do not allow for dissolution of the 2C compounds. Following UHPLC separation, time of flight mass spectrometry will be employed for compound identification. Additionally, a photo diode array detector will be coupled, in-line, to aid in quantification.

Finally, a separate sample introduction technique (direct sample analysis) will be coupled thus providing various methods of analysis and identification of the targeted drugs and impurities which will all be compared and contrasted in this presentation.

MS/MS spectra were used to determine the fragmentation patterns; these fragmentation patterns were observed for nineteen standards. The first fragment for every compound was a loss of 17 amu, representing the loss of the -NH2 substituent. Halogenated compounds display the loss of a halogen group. While all the MS/MS spectra for the compounds were similar in fragmentation patterns, they also displayed differences that allow the analyst to distinguish which compound is present. These fragmentation patterns, in addition to accurate mass TOFMS, will be used to qualitatively identify street samples and also determine impurities. Discovering the impurities within these compounds and raising awareness of the dangers of consuming drugs from incompetent synthesis may prevent future overdoses and health complications and diminish the market for unsafe illegal drugs.

Practical Quality Measures in Drug Chemistry

Jim Wesley - Monroe County Crime Lab, Rochester, NY

Abstract:

Scope: With increased pressures to both decrease case backlog and improve result quality, labs are brainstorming to find solutions that will not require any additional expenditures. We look at several novel procedural innovations that can be implemented without utilizing additional resources.

Method
Identification of pharmaceuticals can be time consuming. This is especially true when the case contains hundreds of tablets of many types. The SOP usually requires a complete tabulation of all evidence and that means hand counting all tablets. The possibility of a miscount and an incorrect result is ever present. The resulting CAR can have serious consequences to both employee and lab. We have established a database of tablet imprints that is very useful in checking case tablet counts. In addition, it can be used to help establish aggregate weight charges for those controlled substances that are associated with aggregate tablet weight. In the absence of this, the lab can use an average of 10 tablets to estimate the number of total tablets present. A simple validation of estimated tablets using the 10 tablet average compared to the actual count may be able to allow reporting of approximate number of tablets present. For cases requiring complete counting, the use of an inexpensive pharmaceutical tablet tray counter is a real time saver.

For example: A green round tablet with a TEVA 833 imprint has been identified as 1 mg Clonazepam. Fifteen cases tested over five years representing several hundred tablets of this type produced the following tablet averages: 167, 169, 167, 169, 170, 172, 168, 164, 167, 172, 167, 167, 172, 170, and 169 mg. Note the very tight average range of 164-172 mg, Mean: 168.7 mg. Using the whole data set, a case containing 500 tablets would be estimated to contain from 490-514 tablets. This database is very useful in catching transposed numbers in a report as opposed to determining an exact count. For example: *Clonazepam was identified in the 63 tablets, aggregate tablet weight; 6.0 g*. 63 tablets have an actual weight range of 10.3-10.8 g. 36 tablets have a weight range of 5.9-6.2 g. The correct tablet count is actually 36 and not 63! The current ***Tablet Imprint Database*** will be distributed.

Use of Microsoft Word Auto Text

Use of ***MS Word Auto Text*** can save a significant amount of time when typing complex drug names or mandatory descriptors and will reduce typos in typed reports and subsequent delays in technical review. In Microsoft Word 10: Select File > Options > Proofing > Autocorrect Options “Replace” _____ “With”_____. When I type “ID" and press ENTER, the phrase ***Identifications were made using Gas Chromatography/Mass Spectrometry*** is automatically inserted. There is no limit to the length of the insertion.

Other useful time saving techniques that improve overall quality will also be discussed. Useful time saving suggestions from the attendees are welcome.

Detecting, Identifying and Sourcing Counterfeit Drugs

Pauline E. Leary, M.S. - Graduate Center of the City University of New York, 365 Fifth Avenue, New York, New York 10016

John A. Reffner, Ph.D. - John Jay College of Criminal Justice, 524 West 59th Street, New York, New York 10019

Abstract:

Counterfeit drugs are a significant threat to public health and safety. The problem is complex and integrates matters of law, science, criminal justice, public health and public policy. The nature of these goods as well as factors that streamline global trade have significantly increased their risk to the American public. The use of unregulated internet pharmacies to fill prescriptions and for the purchase of over-the-counter medications is especially challenging. These pharmacies are difficult to regulate and frequently deliver counterfeit drugs or drug products. Lifestyle drugs like Viagra® and Cialis® are especially susceptible in these situations, but other types of counterfeit pharmaceuticals are also a risk to the American consumer. Recently, a counterfeit version of the cancer medication Avastin® entered the drug-supply chain of the United States. The Wall Street Journal reported in July 2012 that Canada Drugs, an internet pharmacy and drug wholesaler, sold counterfeit cancer treatments from India, Turkey and other countries directly to United States doctors who administer the drug to their patients. This is significant because counterfeit drugs may contain the wrong active pharmaceutical ingredient, the correct active pharmaceutical ingredient in the wrong dose or no active ingredient at all.

If an appropriate response to the counterfeit-drug problem is to be presented, the ability to detect, identify and source these goods is a fundamental requirement. Methods used must be reliable and meet the standards for admissibility of scientific evidence in United States courts. This is a challenge to the field of forensic science because methods used to identify illicit drugs are not always appropriate for identification of counterfeit drugs. For example, detection, identification and quantitative analysis of the active pharmaceutical ingredient of a drug product are routinely used by the forensic scientist to analyze drug samples. When analyzing counterfeit drugs, however, this may only be the first step in the analytical workflow to identify the drug product as counterfeit. Sourcing these samples is an even more difficult task. A review of the forensic science literature with regard to counterfeit goods discusses the value of being able to establish provenance when a counterfeit good is analyzed by the forensic scientist. However, although attempts were successful in some specific instances, a method for the routine analysis of counterfeit goods or drugs to determine provenance has not yet been described.

This presentation will review results of analytical testing performed on counterfeit drugs to detect, identify and determine source or origin. Methods and technologies used include toolmark analysis, vibrational spectroscopy and gas chromatography-mass spectrometry. The discrimination potential of methods used, as well as advantages and limitations of each method for the detection, identification and sourcing of counterfeit drugs will be reviewed.

Rapid Screening of Synthetic Cannabinoids and Plant Material Substrates Using Portable Fluorescence-Free Raman Spectroscopy

Patricia Diaz, Michael Kayat, and Jean Vincenti - Field Forensics, Inc. 1601 3rd Street South, St. Petersburg, FL 33701

Abstract:

Synthetic cannabinoids are sold in the form of smokable herbal incense blends, essentially dried plant materials sprayed with these compounds, and are advertised as a “legal” alternative to marijuana [1]. New forms of these and other synthetic designer drugs, produced by simple organic transformations, are continually being introduced to evade legislative efforts to stop synthetic cannabinoids’ proliferation.

The continued growth of this “emerging drugs” market is contributing to the already significant backlogs at forensic laboratories. A rapid screening method for synthetic cannabinoid standards and plant substrates is presented using a portable Raman spectrometer with excitation at 1064 nm as a complement to other techniques to improve throughput. Raman Spectroscopy is a Category A analytical technique according to SWGDRUG guidelines, as it “yields structural information that will provide sufficient selectivity that generates the highest discriminating capability [2].” It is also non-destructive, so evidence is preserved, can be used to analyze samples within glass and plastic containers, does not require sample preparation, and is not affected by the presence of water in a sample, unlike IR spectroscopy. Other techniques require the extraction of the synthetic cannabinoids present on the surface of the plant materials followed by lengthy GC-MS with typical run times of up to 45 min [3,4]. When sampling plant materials directly, without prior preparative steps, a significant consideration is the potential for a fluorescence signal to be generated at excitation wavelengths between ~275 nm-975 nm, which can completely overwhelm the Raman bands. Traditionally, FT-Raman with an excitation wavelength at 1064 nm has been used to suppress fluorescence in samples that exhibit this phenomenon at shorter wavelengths such as 785 nm, but drawbacks include cost, lack of portability, moving parts, long analysis times, etc. However, due to recent technological advances stemming from the telecommunications industry, a hand portable, dispersive Raman instrument with 1064 nm excitation wavelength can be used to very effectively build spectral libraries and identify unknown samples in the field while minimizing fluorescence.

Analysis results will be presented for several synthetic cannabinoids such as cylcohexylphenols, naphthoylindoles, dibenzopyrans and phenylacetylindoles, as well as various plant materials.

References:

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[4] Tambasco, A. J. "Emerging trends in synthetic drugs." Presentation from: Emerging Trends in Synthetic Drugs Workshop, May 1, 2013.

***Colorimetric Analysis of Salvia divinorum Utilizing Ehrlich's Reagent**

Rhiannon R. Carter, M.S., Keri LaBelle, M.S., Lisa Tozier, M.S., Brian Cawrse, M.S., Adam B. Hall, Ph.D., Biomedical Forensic Sciences Program, Boston University School of Medicine, 72 E. Concord St. R806, Boston, MA 02118

Abstract:

Salvia divinorum is an herb from the mint family, Lamiaceae. The Salvia genus contains a variety of herbs that are commonly known as sage. S. divinorum originates from Oaxaca, Mexico and is known to play a role in spiritual ceremonies of the Mazatecs due to the herb's psychoactive properties. Given its hallucinogenic properties and ease of access via the Internet, this herb has a high potential for abuse. The legal status is not currently well defined, as the regulation of S. divinorum is handled on a state-by-state basis in the USA.

The active component in S. divinorum is salvinorin A, a non-nitrogenous diterpene that is a highly selective kappa opioid receptor (KOR) agonist, is reported to be the most potent naturally occurring hallucinogen. Salvinorin A is consistent with other KOR agonists, which produce effects such as sedation, analgesia, inhibition of GI function, aversion, and depression.

Characterization and analytical detection methods have been described for salvinorin A to include: UV/Vis, HPLC, LC/MS, GC/MS, TLC, FTIR, X-Ray crystallography, and NMR. However, these techniques require extraction of the active component from the leaves of S. divinorum and frequently require analysis on complex instrumentation. The development of a quick, presumptive field test for the analysis of S. divinorum, salvinorin A, and other salvinorin derivatives will allow for discrimination from unrelated plant material and drug paraphernalia.

Our current research has shown that Salvia divinorum produces a unique color result upon reaction with Ehrlich's reagent (p-dimethylaminobenzaldehyde). This colorimetric assay is sensitive to 50 µg of salvinorin A standard, and as little as 1 mg of plant material can be used to produce a colored result. The colorimetric assay is presented along with the data supporting the mechanism of the formation of the colored species in solution. The mechanism is proposed to proceed via electrophilic aromatic substitution at the furan ring of salvinorin A.

*** Collegiate Competition**

Northeastern Association
of
Forensic Scientists

Determination of Four NBOMe Designer Drugs Using ESI-LC-TOF-MS and ESI-Ion-Trap

Ada Kong – Northeast Laboratory

Abstract:

Recently, law enforcement officers have encountered designer drugs called NBOMe (2,5-dimethoxyphenyl-N-(2-methoxybenzyl)ethanamine). NBOMe compounds are psychoactive drugs. These illicit drugs were linked to several hospitalizations and deaths. NBOMe compounds are in the phenethylamine class of drugs and share the same core phenethylamine structure. The chemical structures of NBOMe are similar to 4-bromo-2,5-dimethoxyphenethylamine(2C-B), which is a Schedule I hallucinogen. The Chemical Evaluation Section of the Office of Diversion Control at the Drug Enforcement Administration determined that 25I-NBOMe (2-(4-iodo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine), 25C-NBOMe (2-(4-chloro-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine) and 25B-NBOMe (2-(4-bromo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine), are analogues of 2C-B. The Controlled Substances Analogue Enforcement Act of 1986 states that any person using an analogue, or derivative version of a drug can be prosecuted as if that person were using the original drug.

NBOMe drugs are typically found in powder form or impregnated in blotter paper. These drugs present challenges to forensic chemists who try to identify them because of lack of known spectra libraries or the presence of multiple components in low concentrations. This presentation will demonstrate techniques of identifying four NBOMe compounds, 25I-NBOMe, 25C-NBOMe, 25B-NBOMe and 25D-NBOMe (2-(2,5-dimethoxy-4-methylphenyl)-N-(2-methoxybenzyl)ethanamine), in blotter papers. The experiments were performed using an electrospray ionization liquid chromatography time-of-flight mass spectrometer (ESI-LC-TOF-MS) and an electrospray ionization ion trap mass spectrometer (ESI-ion-trap-MS).

Toxicology Abstracts

The Practical Applications of LC/MS TOF Screening for Drugs

Robert DeLuca - CT DESPP Controlled Substances/Toxicology Laboratory, 278 Colony Street, Meriden CT 06451; Mary Jane Masih, Westchester County Dept.of Labs and Research, Valhalla, NY

Abstract:

Screening for the presence or absence of drugs is of paramount importance in determining cause and manner of death for postmortem toxicology. Driving under the influence of alcohol and other drugs continues to be a problem in keeping our roadways safe. Sexual assaults involving drugs and emergency room visits for suspected overdoses point to a need for a comprehensive, fast turnaround time toxicological analyses for drugs and other poisons. Traditional screening for drugs involved combinations of color tests, immunoassays, GCMS, LCMS and other specific tests, followed by confirmatory testing when needed. GCMS and LCMS have their limitations. The use of HPLC coupled with high resolution accurate mass is another way of screening for drugs.

This presentation will cover sample preparation, instrument conditions, and in interpreting peak chromatograms. It will use actual casework on blood and urine samples as examples of searching for targeted and non-targeted drugs.

DART-MS for rapid, preliminary screening of urine for DMAA

Kendra J. Adams[1], Ashton D. Lesiak[1], Colin Henck[1], Robert B. Cody[2], Marek A. Domin[3], A. John Dane[2] and Jason R. E. Shepard[1]*

[1]Department of Chemistry, University at Albany, State University of New York (SUNY), 1400 Washington Ave., Albany, NY 12222, USA
[2]JEOL USA, Inc., 11 Dearborn Rd, Peabody, MA 01960, USA
[3]Mass Spectrometry Center, Merkert Chemistry Center, Boston College, 2609 Beacon Street, Chestnut Hill, MA 02467-3808, USA

Abstract:

1,3-Dimethylamylamine, known as DMAA, is a stimulant that has gained recent attention due to its use as a doping agent and an ingredient in workout supplements. Its increased prevalence on the market has resulted in a documented rise in emergency visits and calls to poison control centers, and has also been associated with instances involving cerebral hemorrhages and fatalities of users. Due to emerging information about health risks of this drug, DMAA has been under scrutiny by several governments, including Australia, Canada, the U.S., and New Zealand, where it has been identified as an abused substance. Here, we report an ambient ionization mass spectrometry method, direct analysis in real time-mass spectrometry (DART-TOF-MS), used for the detection of DMAA in multiple nutritional supplements as well as directly in unprocessed urine. Solid

nutritional supplements were tested directly as a solid powder or pill, without any preparation whatsoever. In addition, a volunteer ingested the supplement Ripped Juice EX2, containing an unknown amount of DMAA, with urine collected and tested for the presence of the drug. Urine samples were tested directly by DART-MS without any preparations and positively identified the drug over 48 h. Additionally, the raw urine samples were processed in two ways, followed by DART-MS analysis. First, the DMAA was liquid-liquid extracted from the urine with methylene chloride, testing the extract by DART-MS. Second, the urine was processed using DPX tips prior to DART-MS analysis.

MAA was successfully detected from urine in all three sets of samples, with liquid-liquid and DPX extractions giving the most robust responses, as compared to the neat samples. The three processing methods provide an interesting decision point, providing instantaneous analysis with no sample preparations versus more robust responses related to increased levels of sample preparations. Ultimately, DART-MS proved useful in a rapid screening of DMAA in urine samples and demonstrates the technique as a viable preliminary method of analysis of drugs in biological matrices.

Physiology of Energy Drinks

Jim Wesley - Monroe County Crime Lab, Rochester, NY

Abstract:

Energy drinks have become a $7B annual business, and now exceed sports drink sales! We review the history, marketing and use of energy drinks using the authors 2002 Article ***the Red Bull Reality*** as a starting point. We then focus on the effects of their primary ingredients; caffeine, taurine and glucuronolactone. Much of the focus regarding energy drink effects relates to caffeine and so we begin the discussion with a comparison of the caffeine levels in energy drinks to published caffeine levels in common beverages.

The real effects however relate to the taurine. This sulfur containing non-essential amino acid, (which is vital for cats) acts directly on the heart, increasing stroke volume and cardiac output at rest without increasing blood pressure. At doses exceeding 3 grams per day, it acts very similarly to digoxin, in fact there has been discussion regarding its use in congestive heart failure. Featured articles in J. Amino Acids will be presented.

We continue the discussion with a focus on “speed balls”, alcohol/energy drink combinations which remain popular in bars despite the removal of the energy drink component from commercially manufactured products.

We conclude with a review of recent cases involving hospitalizations and deaths from the use of these beverages and a caution from the European Food Commission.

Uncertainty of Blood Alcohol Concentration (BAC) Results as Related to Instrumental Conditions: Optimization and Robustness of BAC Analysis Parameters

Haleigh A. Boswell and Frank L. Dorman, PhD, The Pennsylvania State University, 107 Whitmore Laboratories, University Park, PA 16802

Abstract:

Analysis of blood alcohol concentration is a routine analysis performed in many forensic laboratories. This analysis commonly utilizes headspace-sampling, followed by gas chromatography combined with flame ionization detection (GC-FID). Studies have shown several "ideal" methods for instrumental operating conditions, which are intended to yield accurate and precise data. Given that different instruments, sampling methods, application specific columns and parameters are often utilized, it is less common to find information on the robustness of these reported conditions. A major problem can arise when these "ideal" conditions may not also be robust, thus producing data with higher than desired uncertainty or inaccurate results.

The goal of this research is to incorporate the principles of quality by design (QBD) in the development of BAC instrument parameters, thereby ensuring that minor instrumental variations, which occur as a matter of normal work, do not appreciably affect the final results of this analysis. This presentation will discuss both the QBD principles as well as the results of the experiments, which allow for determination of "ideal" instrumental conditions. Additionally, method detection limits will also be reported in order to determine a reporting threshold and the degree of uncertainty at the common threshold value of 0.08g/dL. Finally, differences between pressurized loop headspace systems and volumetric headspace systems will be discussed, comparing and contrasting these two different types of analytical instruments.

Automated Sample Preparation for Gas Chromatography / Mass Spectrometry (GC/MS) and Liquid Chromatography / Mass Spectrometry (LC/MS)

John Edleman - GERSTEL, Linthicum, MD

Abstract:

The main focus is on automated sample preparation for Gas Chromatography / Mass Spectrometry (GC/MS) and Liquid Chromatography / Mass Spectrometry (LC/MS).

GERSTEL delivers automated solutions focused on the needs of the customer. As a specialist for automated sample preparation and sample introduction, GERSTEL is able to provide custom solutions, including complete solutions that include GC/MS and LC/MS with integrated software control. The innovative MAESTRO software enables intuitive control and flexible, efficient operation for R&D or routine laboratories. Your daily analysis tasks are set up using just a few mouse-clicks.

Ritualistic Herbs

Jim Wesley - Monroe County Crime Lab, Rochester, NY

Abstract:

Since 2010, synthetic drugs have been a focus of the media, law enforcement, hospital emergency departments and rehabilitation centers. Toxicology labs and crime crimes have been at the forefront of synthetic drug identification. In an attempt to control the use of synthetic drugs, laws were enacted in 2011 and 2012 to restrict these compounds with limited success.

Natural intoxicants, although available, have escaped our interest, yet I find them very interesting. As laws restrict synthetic drugs, the use of natural intoxicants, which have unique structures and properties, may dramatically increase. In my opinion, the unique structures of these natural intoxicants will make them difficult to control.

We begin with a look at the 1994 Dietary Supplement Health and Education Act (DSHEA) which set guidelines and loose regulations for “natural products” that are non-controlled.

We then focus at the history of the ritualistic use of these intoxicants by native peoples. We will then discuss availability of these substances and their use as modern intoxicants. We will cover desired effects, side effects, toxicity and detection. Featured intoxicants will include: Absinthe, Belladonna, Betel, Caapi, Dragons Blood, Henbane, Hops, Jimsonweed, Kava, Mugwort, Nutmeg, Sweet Flag, Velvet Bean, White Copal, Valerian, Yohimbe, and Yopo. As usual, the PowerPoint will feature an excessive number of slides containing great images!

A table containing the herbal names, psychoactive chemicals present, and MS ions will be provided.

Postmortem Redistribution - Mechanisms and Consequences

Robert H. Powers, Ph.D., DABFT; University of New Haven, West Haven CT, and CT DESPP Controlled Substances/Toxicology Laboratory, 278 Colony Street, Meriden CT 06451

Abstract:

Postmortem Redistribution (PMR), is a well-recognized phenomena that may affect the concentration of drugs in samples collected after death. Examples of PMR have been extensively documented in the forensic literature for many years, nevertheless, the occurrence and magnitude of PMR remains unpredictable at the level of the individual case. This presentation is designed to review and explore the basis of PMR, specifically, the mechanisms by which PMR can occur, and to underscore the extent to which drug values from postmortem samples may, in some cases, be significantly misleading. The potential consequences of PMR, in terms of the possibility for misinterpretation of the role that a drug, or combination of drugs may have played in a death can be significant. Therapeutic levels of drugs can, as a function of PMR, generate concentrations in postmortem samples that would be considered toxic or even lethal if truly representative of antemortem levels. While the potential for a drug to exhibit PMR has been suggested to be generally correlated to Vd, that parameter fails to be reliably predictive. The mechanisms by which drugs can be concentrated during life

in a tissue-specific manner are reviewed from a biochemical perspective, particularly with reference to the mechanisms for eventual diffusion in the postmortem interval. Specific case examples are discussed to illustrate some of the problems associated with interpretations of postmortem drug concentrations when PMR may be affecting those levels. Recent attempts to provide a quantitative, or statistically-based framework for evaluation of postmortem drug concentrations, and "back-extrapolation" are discussed.

Method Development and Validation in Forensic Toxicology

Amanda L. Arntson, MSFS, The Center for Forensic Science Research and Education, and Barry K. Logan, PhD, NMS Labs/The Center for Forensic Science Research and Education

Abstract:

Expectations for the accuracy, reproducibility and robustness of analytical methods used in forensic toxicology have increased over recent years. This has led laboratories to spend additional resources on validation to ensure that complete documentation is in place, demonstrating the capabilities and limitations of assays they use for analysis of forensic toxicology casework. There is a broad range of approaches to method validation, and the Scientific Working Group on Toxicology (SWGTOX) has recently proposed a general guidance document detailing the parameters of method validation. This presentation will examine the components of a defensible method validation approach, and review some of the options available to comply with the SWGTOX standards. Validation of quantitative methods should include at a minimum, standard verification, determination of the limit of detection and quantitation, the linear range, within and between run accuracy and precision (which includes an assessment of robustness), an assessment of the sensitivity and specificity of the method in terms of its ability to distinguish between positive and negative specimens, an assessment of the methods vulnerability to interference from the matrix or from other related analytes, operating performance of the method with respect to recovery, and the stability of the target analyte under different collection and storage conditions, and any associated bias in the method. In addition, methods need to undergo some limited re-validation when any key element in sample preparation or the analytical platform is changed to demonstrate accurate results are still obtained. This presentation will review several approaches to these experiments and how to present and evaluate the data.

2013 Update from the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG)

Robert H. Powers[1], Ph.D., DABFT, and Jack Mario[2], MS; [1]University of New Haven, West Haven CT, and CT DESPP Controlled Substances/Toxicology Laboratory, 278 Colony Street, Meriden CT 06451; [2]Suffolk Co. Crime Laboratory Hauppauge, NY 11788

Abstract:

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) was formed in 1997 in a joint effort between the U.S. Drug Enforcement Administration (DEA) Office of Forensic Sciences and the Office of National Drug Control Policy (ONDCP). The mission of SWGDRUG is to recommend minimum standards for the forensic examination of seized drugs and to seek their international acceptance. This presentation will provide attendees with an update on SWGDRUG activities during the year 2012 and currently in 2013. Recent

activities include version 6 of the Recommendations, considerations for the use of reference materials (including verification) and guidance regarding new and emerging materials, supplemental documents addressing measurement uncertainty, and reporting examples. Current issues being considered by the core committee include online resources for drug analysis training, and criteria for designation of drug analogues. The SWGDRUG mass spectral library remains an extensively utilized resource within the forensic community, and current status as well as future plans will be reviewed.

The SWGDRUG core committee includes representatives from federal, state, and local law enforcement agencies in the United States, Canada, Brazil, Great Britain, Germany, Austria, Switzerland, Australia and Singapore. Organizations represented include the European Network of Forensic Science Institutes (ENFSI), the Academia Iberoamericana de Criminalistica y Estudios Forenses (AICEF), the Asian Forensic Science Network (AFSN), and the United Nations Office on Drugs and Crime (UNODC). The core committee includes forensic science educators, and representatives from forensic science organizations across the Unites States, including the American Society of Crime Laboratory Directors (ASCLD), the American Academy of Forensic Sciences (AAFS), The American Society for Testing and Materials (ASTM) and the National Institute of Standards and Technology (NIST).

Biology Abstracts

Field-Deployable Rapid DNA Analysis: Fully-integrated, Fully-automated Generation of Short Tandem Repeat Profiles from Buccal Swabs

Eugene Tan, PhD – NetBio, 830 Winter Street, Waltham MA 02451

Abstract:

This presentation will describe development of a Rapid DNA Analysis system consisting of a modular platform that allows customization to perform a wide range of nucleic acid analyses. Independent microfluidic modules developed include those for DNA purification, DNA quantitation, highly multiplexed amplification, DNA sequencing, electrophoretic separation and detection, and related control, analytical, signal processing, and expert system profile determination software. The system consists of a fully automated instrument and a single accompanying BioChipSet cassette that can be used by non-technical personnel in laboratory, office, or field-based settings while dramatically reducing the time to perform STR analysis. In this presentation, we will summarize the application of microfluidics to forensic molecular biology and our success in using the fully integrated system to generate CODIS-quality DNA profiles in 84 minutes from buccal swabs without human intervention.

The Rapid DNA Analysis System is operated by inserting five buccal swab samples into a BioChipSet Cassette, placing the BioChipSet into the instrument, and then closing the door to initiate the run. The instrument provides all the subsystems required for the completion of STR analyses, including the power, thermal cycling, pneumatic, optical, ruggedization, process control, and computer subsystems. The instrument interfaces to the biochipset using a number of features, including a pneumatic manifold (to allow fluids to be driven), thermal features (to maintain appropriate temperatures during PCR and electrophoresis), optical paths (to allow excitation and detection of separated STR fragments), and electrical connections (to allow electrophoresis). The system is based in part on microfluidic technology and has several critical features:

- The biochipset contains all reagents on-board. The user neither loads the instrument nor the biochipset with reagents. Several reagents are lyophilized (e.g. amplification reaction mix) and others are in liquid form (e.g. purification reagents).

- The biochipset is closed: each buccal sample is processed through its own sealed processing path and samples and reagents do not have contact with the instrument itself.

- The biochipset is a single consumable part; the operator has nothing to connect; no washing or opening is required, minimizing the possibility of run-to-run contamination.

- Buccal swabs lock into separate purification positions so that once loaded they cannot be move to another location. An RFID chip in the swab cap is detected by the instrument and used identify the swab location within the BioChipSet.

- The instrument is ruggedized to MIL-STD 810F for shock and vibration. This allows it to be moved within the forensic laboratory, transported for use outside of the laboratory, or used in a police station or field-forward setting. Instrument auto-calibration readies the instrument for use within 15 minutes of setting it up.

- The instrument contains an on-board computer and touch screen monitor for interfacing with the operator, and the instrument's wireless, USB, and Ethernet connectivity options can be configured to user requirements. Also based on user requirements, the system includes an expert system for conversion of electrophoretic traces to CODIS/NDIS compatible profiles and .cmf output, GPS-tagging of data products with time and location data, and an internal database to store instrument-generated profiles.

- The system has on-board an Expert System software that automatically process all data and designates alleles to generate STR profile with no analyst intervention or review.

Description of the instrument design, the processes conducted in automated processing, and data characterizing the output generated from the fully integrated multiplex STR instrument-biochipset-expert system format will be presented.

Rapid Human Identification: Evaluation of the RapidHIT™ 200

Frank Wendt and Mitchell Holland, Penn State University, Forensic Science Program

Abstract:

The forensic science community has been effectively using traditional methods of Short Tandem Repeat (STR) analysis for more than twenty years, through PCR amplification and gel or array-based capillary electrophoresis. Emerging technologies offer the ability to advance the speed and mobility of the STR analysis process. One such platform is the RapidHIT™ 200 system from IntegenX, which combines the cell lysis, DNA purification, quantification, amplification, and capillary electrophoresis steps into one bench-top unit that generates an STR profile from cheek swabs in less than 90 minutes. The instrument is currently capable of providing investigative leads through the analysis of reference samples. When employed in police stations, rapid testing results can be compared to local databases of unsolved casework, or can be compared, if acceptable in the relevant jurisdiction, to state and federal databases. We have evaluated the RapidHIT™ to determine if the instrument is ready to be adopted by the forensic community. Contamination and reproducibility studies illustrated the platform's ability to outperform traditional bench top methods when dealing with freshly collected (pristine) samples. Analysis of baseline noise resulted in an analytical threshold (AT) of 900-1500 RFU for detection of true allelic peaks, with typical peak heights well over 12,000 RFU. Peak height ratios for profiles at different levels of intensity were used to determine a stochastic threshold (ST) to guard against falsely reporting homozygote profiles. Finally, a study was conducted to assess how the RapidHIT™ 200 handles aged samples, to determine potential impacts of storing samples prior to rapid DNA analysis.

***Genomic DNA Isolation from Amplified Product for Recursive Genotyping of Low-Template DNA Samples**

Joseph R. Iacona, Amy N. Brodeur and Catherine M. Grgicak - Boston University School of Medicine, 2013

Abstract:

Biological evidence may contain any number of cells in any proportion. Extreme low-template DNA samples are often very difficult to interpret due to complex signal or peaks which may be indistinguishable from baseline noise. Current solutions focus on increasing the amount of amplicon detected by adjusting PCR cycle number or capillary electrophoresis injection parameters. Consensus profiling is an additional option. However, the aforementioned solutions are often not helpful for extreme low-template samples due to the high occurrence of allelic drop-out. Additionally, PCR is a destructive technique that causes one amplification to completely exhaust this type of sample, making further typing and analysis impossible. Therefore, a technique that allows for the re-generation of a DNA template in order to amplify it multiple times would be an extremely useful tool.

This study outlines the development of a method that allows for the recursive amplification of a DNA sample. Amplification was performed using biotinylated primers for an STR locus and the resulting product was cleaned using streptavidin-coated magnetic beads to sequester the amplicons. Subsequent centrifugal filtration was used to remove the remaining PCR components, thus isolating the original genomic DNA. Re-amplification was then successfully performed at a different STR locus.

Though successful, multiple run-throughs of the method indicated retention of signal from the original amplification as well as significant genomic DNA loss during the process. This study outlines experiments seeking to characterize the cause(s) of these imperfections in order to effectively direct method optimization. A computer generated dynamic model was also created and used to simulate the recursive amplification process to assist in development. When optimized, it is expected that recursive amplification can significantly reduce the difficulties associated with low-template DNA analysis and eradicate the concept of an 'exhaustive' DNA sample.

*** Collegiate Competition**

Evaluation of Direct PCR Amplification Using Swabs and Washing Reagents

Hallie D. Altshuler & Reena Roy - Pennsylvania State University, 107 Whitmore Laboratory, Universtiy Park, PA 16802

Abstract:

The objective of this study was to generate DNA profiles from body fluids deposited on different swab substrates. These samples were pretreated with purification reagents or extraction buffers prior to amplification with direct and non-direct amplification kits.

Saliva samples from living donors and blood samples from deceased individuals were deposited on Pur-Wraps® sterile cotton tipped applicator, Pur-Wraps® Foam Swab, MiniPax®, PurFlock Ultra Flocked swab, FAB-

swab, Fitzco SPIN-EZE™ Push-off™ swab, and Bode SecurSwab™. These devices were kept at room temperature for varying lengths of time ranging from one day to approximately one year. 1.2mm punches were obtained from each swab substrate containing body fluids and washed with PunchSolution™, SwabSolution™, Prep-n-Go™ Buffer, ECS™ wash buffer, and FTA™ reagent and amplified using various amplification primers obtained commercially. A 1.2mm punch of a negative control of each type of swab was also treated similarly.

Autosomal STR loci and the amelogenin gender locus were amplified using PowerPlex® Fusion reagent kit from Promega Corporation and AmpFℓSTR® Identifiler® Direct, Identifiler® Plus and Identifiler® amplification kits from Applied Biosystems®-Life Technologies (AB). In addition, male body fluids were amplified using PowerPlex® Y23 System from Promega Corporation. Substrate types, amount of reagents needed, and, if necessary, amplification parameters were varied in this study to detect autosomal and Y-STR DNA profiles. Analysis of the amplified product was performed by capillary electrophoresis injection on the AB 3130xl Genetic Analyzer. The generated DNA profiles were analyzed using GeneMarker® HID Software Version 2.2.0 from SoftGenetics®.

Autosomal and Y-STR profiles were generated successfully from the swab substrates containing blood and saliva. Concordant profiles were obtained within and between all amplification kits.

*** Collegiate Competition**

Comparison of Reducing Agents in Differential DNA Extraction

Lauren Cohen and Lawrence Quarino – Cedar Crest College, Allentown, PA 18104

Abstract:

Differential extraction procedures have been utilized on sexual assault evidence for two decades and involve the selective digestion of epithelial cells followed by isolation and digestion of sperm cells. A reducing agent is used to lyse sperm so that DNA from these cells can be isolated. Although several alternatives are available commercially that have increased throughput, many laboratories still use the traditional Chelex® or organic differential extraction method due to problems related to cost and implementation. Recently, a new reducing agent, dithiobutylamine (DTBA), was synthesized from L-aspartic acid and found to be far superior in reducing disulfide bonds in aqueous solution when compared to several reducing agents including DTT (J Am Chem Soc 2012;134:4057-9). Reduction of disulfide bonds on the membrane of sperm cells is necessary to isolate sperm DNA in these procedures. As a result of this research, a pilot study was conducted to see if male DNA profile quality can be improved by comparing the use of DTBA in a differential extraction procedure not only to DTT but to other reducing agents as well.

Simulated post-coital swabs were created by adding 2 μL of neat semen, 1:10 diluted semen, and 1:100 diluted semen respectively to buccal swabs and allowed to dry. Differential Chelex® extraction was performed in triplicate on each set of swabs respectively using DTT, DTBA, beta- mercaptoethanol and Tris (2-carboxyethyl) phosphine.

Each reducing agent tested had a concentration of 1molar and a volume of 7ul of each was used. Extracted DNA was quantified using the Alu-based real-time PCR SYBR green method and appropriate DNA concentrations were amplified using the PowerPlex 16 HS® system. Fragment analysis of amplified products was performed on an ABI Prism 310 Genetic Analyzer and amplicons were genotyped using GeneMapper® ID-X software.

Results from swabs containing neat semen showed no difference in the ability to obtain a sperm DNA profile in differentially extracted samples using DTT, DTBA, or beta-mercaptoethanol. Triplicate swabs using these reducing agents all produced full sperm profiles. No male DNA was detected when Tris (2-carboxyethyl) phosphine was used as the reducing agent. As a result, none of the swabs which used Tris (2-carboxyethyl) phosphine as a reducing agent were genotyped.

The quality of sperm DNA profiles did vary with swabs containing 1:10 and 1:100 diluted semen with the three reducing agents. DTT did not produce any full profiles with swabs containing either 1:10 or 1:100 diluted semen. The male fraction averaged 22 and 12 alleles (out of a possible 26) for the 1:10 and 1:100 diluted semen swabs respectively when using DTT as the reducing agent.

Both DTBA and beta-mercaptoethanol treated swabs produced more complete sperm DNA profiles than DTT. With DTBA, an average of 25 male alleles was detected on the 1:10 diluted semen swabs and two of the three swabs produced full male profiles. With the 1:100 diluted semen samples, DTBA treated samples averaged 16 male alleles although no full profiles were obtained. All three swabs containing 1:10 diluted semen produced full profiles with beta-mercaptoethanol and an average of 17 male alleles were detected on the 1:100 diluted samples. No full male profiles were obtained on the swabs containing 1:100 diluted semen when beta-mercaptoethanol was used as the reducing agent.

Results from this pilot study indicate that both DTBA and beta-mercaptoethanol used as a reducing agent in differential extraction may be superior in developing sperm DNA profiles from male-female mixed samples than the commonly used DTT, particularly with samples containing a small volume of semen.

*** Collegiate Competition**

Detection of Male DNA in the Vaginal Cavity Following Digital Penetration Using Y Chromosome Short Tandem Repeats

<u>Janine M. Kishbaugh</u>, M.S. and Kayla R. Sween M.S. - Cedar Crest College, Allentown, PA 18104

Abstract:

Analysis of rape cases can be challenging if a victim does not report the incident immediately. As the time interval between the rape and report increases the more difficult it becomes to obtain a DNA genotype. The definition of rape, a form of sexual assault, has been recently been modified by the FBI to include digital (finger) penetration. This updated definition of rape is especially important with adolescent and adult female sexual assaults where penile-vaginal penetration does not occur. Y chromosome short tandem repeats (Y-STR) are important in the analysis of rape cases where autosomal short tandem repeat genotypes cannot be obtained. Currently, vaginal

swabs collected up to eight days post-coitus have been found to yield Y-STR profiles. However, sexual assault kits are not commonly collected if only digital penetration is reported.

The purpose of this study is to determine if male DNA can be detected in the vaginal cavity following digital penetration at 1, 6, 12, 24 and 72 hours post penetration. To obtain simulated samples, volunteer male-female couples were asked to abstain from sexual activities for a given period of time prior to collection. The couples participated in vaginal-digital penetration for a discrete period of five minutes. Female participants collected four vaginal swabs prior to penetration as a control and four swabs at the specified time interval post-penetration. Each experimental collection occurred after a new penetration event. The vaginal swabs were then processed using optimized protocols for the greatest detection of male DNA. Five of the eight participating couples produced full profiles one-hour after digital penetration occurred. It was determined that a Y-STR profile can be detected up to 72 hours post-penetration, with some couples resulting in an almost complete profile (21 of 23 alleles), a full profile was obtained when the six genotyping results were compiled. Of the 116 total swabs or 58 swab sets that were collected, DNA profiles were successfully obtained from 54 or 93.2% of swabs sets collected. Results varied by couple and by time interval, the variation was attributed primarily to the time of collection relative to the female menstrual cycle.

In conclusion, male DNA can be detected from vaginal swabs collected following simulated digital penetration. This is specifically significant in adolescent sexual assaults where the detection of any male DNA in the vaginal cavity shows inappropriate conduct occurred. This indicates that sexual assault kits should be collected from all victims of rape even if digital penetration is the only type of rape reported.

Validation of an External Standard Curve for the Quantifiler® Duo DNA Quantification Kit

Christopher Shaw, Kevin MacLaren, Elayne Schwartz, David SanPietro – Westchester County Forensic Laboratory, Valhalla, NY

Abstract:

Human specific qPCR is commonly used in forensic laboratories to provide both human and male DNA concentrations. The Quantifiler® Duo DNA Quantification Kit from Applied Biosystems utilizes the enhanced multiplexing technology of the Applied Biosystems 7500 Real-Time PCR system and v.1.2.3 f2 sequence detection software for providing DNA concentrations from evidentiary samples.

The concentration results generated via qPCR are dependent on the standard curve generated on each run. Thus, the reproducibility of these results is dependent on a consistent calibration curve being generated from run to run. Varying standard concentrations and pipetting errors (both human and mechanical) can result in inconsistent slopes, y-intercepts and R2 values ultimately affecting qPCR results. These inconsistent qPCR results could lead to non-optimal amplification of DNA and poor STR results.

The main purpose for generating a validated standard curve is to reduce variables that can affect a generated standard curve for each qPCR run. Removing these variables will help in producing more consistent results from run to run.

Currently, 16 standards are prepared for each run. The elimination of these standards reduces the amount of Quantifiler® Duo reagents consumed for each run. There are also times when the standard curve on a qPCR

run does not work. Not only are the Quantifiler® Duo reagents wasted for the whole plate, but DNA evidence in the form of the extract is lost. Another benefit is by removing the 16 standards from each run, 16 extra casework samples can be included on a plate, thus increasing throughput for the laboratory.

This presentation will concentrate on the validation of a standard curve for the laboratory and will also discuss problems encountered with standard curves that are prepared each time a plate is run for qPCR. This validation will help increase sample throughput, cut costs for the laboratory and provide better reproducibility.

A Comparison of Percent Shared Alleles in Related and Unrelated Population Groups as an Aid to

Understanding Coincidental Matching in DNA Profile

Stephanie Tedeschi and Heather Miller Coyle - University of New Haven Forensic Science Department, 300 Boston Post Road, West Haven, CT 06516 USA

Abstract:

A study was performed to look at numbers of shared alleles in specific forensic situations. Initially I was instructed to choose an adjudicated case to organize and evaluate. The case chosen was a Canadian sexual assault case. This case described incidents of incestuous rape resulting in 2 separate occasions of pregnancy and therapeutic abortions. DNA testing was performed on the uterine contents to determine a paternal relationship to victim's relative. Based on this case, to further expand the study to related individuals, relevant laboratory work was conducted to look at shared DNA between unrelated and related populations. Samples were collected from 19 family members (including myself) and the DNA process from collection to profile generation was completed. 100 samples of anonymous unrelated individuals collected from training exercises in the University's Forensic DNA laboratory were compared to published allele frequencies of 3 different racial groups and again to the 19 samples of related individuals. From this data collection and comparison, a statistical analysis was done to establish the amount of DNA shared between populations of related and unrelated individuals. The presentation includes statistical analysis for the case, allele frequencies for each data set and a family pedigree.

Keyword: shared DNA, allele frequencies, relatedness, kinship, comparisons

Development of an Innovative DNA Quantification and Assessment System

A. Holt, J. Mulero, J. Shewale, J. Muehling, J. Benfield, S. Olson, K. Fenesan, R. Green, M. Danus - Human Identification Group, Life Technologies, Foster City, CA 94404

Abstract:

Recently introduced STR kits are highly sensitive, robust and discriminating thereby generating useful STR profiles from previously untypeable samples. Such samples often have low quantity and/or degraded DNA, may contain PCR inhibitors, and, in sexual assault samples, a high quantity of female DNA compared to male DNA. These factors can make it difficult to decide whether to continue with STR analysis, which STR kit to use and how much DNA to add to the STR amplification reaction for obtaining a useful profile on the first attempt. Thus, there is a need for a highly sensitive,

robust, and faster method for the assessment of DNA extracts. We describe a new DNA quantification and assessment kit to provide better correlation between the DNA sample and resulting STR profile. This next generation DNA quantification and assessment kit has high sensitivity (sub-pg level), for both the human and male targets, higher inhibitor tolerance to match next generation STR kits and a unique metric for the determination of DNA quality; Degradation Index. The Degradation Index is a quantitative measure of the degree of DNA degradation, useful for the determination of how much DNA to add to the STR reaction and which STR kit to proceed with. The time required to perform amplification has been reduced, to less than one hour. Furthermore, the standard curve generation protocol is optimized to provide consistent results. We have successfully used this system as a decision making tool to obtain complete STR profiles from challenging samples. These samples include trace DNA samples, highly degraded DNA samples, low quantity of male DNA in a high level of female DNA as well as samples contaminated with PCR inhibitors. Data demonstrating how this new quantification and assessment kit provides valuable sample quantity and quality information for making critical decisions in the STR workflow will be presented, illustrating how this approach can facilitate enhanced efficiency and first pass success rates.

YSTRs: The Y and the How?

Cristina P. Somolinos, MLIS, D-ABC - DNA Laboratory, Office of Forensic Sciences, New Jersey State Police

Abstract:

Considerations when searching YSTR haplotypes in the US Y-STR database. Examples will be shown of the different results that may be obtained for the same haplotype in the database, depending on the size of the population subset being ignored. Haplotypes from proficiency tests and the Romanov haplotype will be used to demonstrate.

How to present the resultant statistics.
Explanation of our lab's experience with the use of the database to generate best estimates of the frequency of haplotypes, development of our own worksheet to calculate the Clopper-Pearson/exact interval. Technical concerns: confidence intervals, significant figures, rounding and truncation of calculations in the on-screen results, connection timeouts.

Comparison with YHRD database.

***Forensic Analysis of Cucurbita pepo**

Amanda Chung[1], Nina Theis[2], Dawn Holmes[1]
[1]Western New England University, Springfield, MA
[2]Elms College, Chicopee, MA

Abstract:

DNA profiling has become increasingly important within the field of forensic botany. Molecular patterns from different plants can be used to reconstruct crimes and identify origin(s) of plant material linked to a crime scene. Microsatellites or short tandem repeats (STRs) are frequently used for these analyses because they are highly variable both within and between species and are easily tractable with PCR based analyses. In this study, we analyzed microsatellite variability within the species *Cucurbita pepo* subspecies *texana*. This species was selected because of its high genetic variability and the fact that it is broadly distributed across the North

American continent. The entire genome of *C. pepo* is not yet available, however, 500 different SSR markers have been identified in this species and a number of them are polymorphic. DNA was extracted from 50 different plants, and 30 of the most polymorphic SSR markers were analyzed with both sequence analysis and high resolution melt curve analysis. Several of the SSR markers showed great promise for development of DNA profiling techniques within this species. The loci with the greatest heterogeneity included CMTp26 with 10 different alleles, CMTp176 with 9 different alleles, and CMTp61, CMTp66, and CMTp169 with 6 different alleles. The sequence TC was the STR associated with CMTp26, CMTp176, and CMTp61, and it was repeated 4 to 33 times within these loci. The repeated sequence found in CMTp66 and CMTp169 was GAA and it was repeated 1 to 14 times. The most predominant genotypes for CMTp26, CMTp176, CMTp61, CMTp66, and CMTp169 were 16 repeats (51.2% of the population), 14 repeats (45.2% of population), 6 repeats (36% of population), 9 repeats (46.5% of population), and 2 repeats (15.8% of population), respectively. Based on these results, future DNA profiling studies with *C. pepo* subspecies *texana* should focus on these 5 microsatellite loci.
*** Collegiate Competition**

Examination into the Applicability and Stability of a Single External Calibrator for Forensic DNA Quantification

Michael C. Cicero and Catherine M. Grgicak - Biomedical Forensic Sciences Program, Boston University School of Medicine, Boston, MA 02118, USA

Abstract:

The accurate interpretation of DNA is highly dependent on the proper application of peak height ratio, stutter and analytical thresholds for a specified target mass of DNA. Some state-of-the-art interpretation methods, which assess the probability of drop-out, drop-in and peak height, may also rely on knowing the quantity of input DNA. However, absolute quantification using standard qPCR methods have been shown to exhibit low levels of reproducibility. Previous studies have suggested that the use of multiple serially diluted external calibrators is a major source of this imprecision. Recently, recommendations to use a single validated curve have been proposed since it has been hypothesized that employment of linear parameters obtained from a single validated curve would eliminate variations associated with pipetting and other laboratory processes. However, this is true only if the instrument detection system and optics are stable over significant periods of time.

Therefore, a long-term study which examined quantification variability was performed. Quantification data from 8 extracts, quantified 13 times over a 26 month period were collected and two different methods of sample quantification were examined. The first employed the manufacturer's recommended protocol (MRP), which uses a standard curve, made from serially diluting a previously quantified sample, run on the same plate as the sample. The second method used one validated curve (VC) which was established by running a set of dilution series created using different pipettes, buffer lots, standard lots and plates/runs.

Radar charts, which plot *the sum of squares* for the RPPH1 and SRY quantification values obtained with the VC and MRP methods, show that the VC method exhibited higher reproducibility over the 26 month period. The total area under the curves obtained with the MRP was 6- and 3-fold greater than the VC area for the RPPHI and SRY loci respectively.

Further, examinations into the stability of quantification results between kit lots, run date and instrument calibration dates were performed. When sample concentrations and calibration dates were considered, random error between runs was larger after the first calibration for both methods, indicating instrument calibration can have a significant impact on qPCR reproducibility. Kit lots did not significantly impact the error associated with DNA quantification.

In conclusion, these finding suggest the VC method results in higher quantification reproducibility between runs. However, significant variations were observed post-instrument calibration. As a result, if the VC method is employed, generation of a new curve after instrument calibration, or any other modification to the instrument detection system, is expected to improve quantification reproducibility and stability. No significant drift was observed between calibrations, suggesting the VC did not need to be re-generated between instrument calibrations/modifications. However, given that instrument drift may directly relate to frequency/hours of use, careful examination of the Internal PCR Controls and/or stability studies are recommended.

***Age Determination of Bloodstain Using Enantiomeric Ratios of Amino Acids**

Coleen Spease and Larry Quarino, Cedar Crest College, Allentown, PA 18104

Abstract:

Bloodstains are common forms of evidence found at crime scenes. Their evidential value is immense, such as providing DNA evidence that can lead to identity and information about the manner and direction of deposition. One vexing issue that has eluded forensic scientists for decades is an accurate determination of how long a bloodstain has been deposited on a surface. The primary literature is filled with potential methods suggested to reliably estimate the age of a bloodstain; however no consensus method has emerged. A method to accurately determine the age of a bloodstain has relevance in scenarios where bloodstains are present at a crime scene (or a presumed crime scene) but may have been deposited at a time unrelated to the event being investigated. The ability to provide temporal information about bloodstains may also be helpful in the probative selection of bloodstains for DNA typing purposes.

Amino acids undergo a natural racemization from their native form, L-, to the D-form. This conversion occurs slowly overtime and continues until equilibrium is reached. It has been postulated that until this equilibrium is reached, the ratio between the L- and D- amino acid can be utilized for age determination. Racemization of aspartic acid and glutamic acid in teeth has been utilized to estimate the age of individuals.

This presentation will summarize the work to date of a study designed to use amino acid racemization to determine the age of bloodstains under various environmental conditions. The method developed utilizes hydrolysis of bloodstains with concentrated hydrochloric acid for 24 hours at 100°C followed by neutralization and filtration. Filtrates were examined for the presence of L- and D- aspartic acid, glutamic acid, and serine using chiral chromatography on a LC-MS/MS and chromatographic peak integration was used to determine enantiomeric ratios. Calibration curves and figures of merit (limit of detection, limit of quantitation, and linear dynamic range) will be reported.

*** Collegiate Competition**

***Differential Extraction Using Erase Sperm Isolation Kit - A Validation Study**

Angelina Gaspero, Randee Sedaka, Jennifer Elliott, and Reena Roy Pennsylvania State University and AB-Life Technology

Abstract:

The goal of this research was to validate the Erase Sperm Isolation Kit protocol

The procedure used in this research will impact the forensic science community by exploring ways to improve the differential extraction process, with the intention of generating single source male autosomal DNA profiles from a mixture of semen and female body fluids (for example, vaginal secretions, blood, saliva).

Depending on the case scenario, there is often an overwhelming amount of female cells relative to the number of male cells. Historically, labor intensive and cumbersome differential extraction procedure steps attempt to isolate the male DNA fraction of the spermatozoa from the female cells. Although time consuming, the separation of non-sperm and sperm cells may be incomplete and may generate a mixture of male and female DNA.

The Erase Sperm Isolation Kit from Paternity Testing Corporation uses an enzyme to selectively degrade DNA from non-sperm cells, leaving the pelleted spermatozoa intact. This enzyme does not destroy DNA within the heads of the spermatozoa, which allows for the generation of a single source male autosomal DNA profile.

This study evaluated the effectiveness of the Erase Sperm Isolation Kit using various sample types including; post-coitus vaginal swabs, a mixture of seminal and female body fluid samples deposited on various substrates, and serially diluted vaginal epithelial cells mixed with a fixed volume of seminal fluid. In addition, single source profiles were obtained when fabrics containing mixture of semen and female body fluids were pre-treated with a cold water washing machine cycle and then dried at room temperature. DNA was extracted from the non-sperm and sperm fractions using Erase Sperm Isolation Kit and purified using DNA Investigator kit and BioRobot EZ1. The DNA from both fractions was quantified, normalized to an optimal DNA target, amplified using commercially available PCR Amplification Kits, and run on the 3130 XL Genetic Analyzer.

The results of the study demonstrated that complete male and female autosomal STR DNA profiles could be generated from most of the specimens containing mixtures of seminal and female body fluids using the Erase Sperm Isolation kit and associated protocol. Mixture profiles containing both male and the female DNA were observed when some of the serially diluted samples containing overwhelming number of female non-spermatozoa cells were mixed with a much smaller amount of semen.

The method described above is cost effective, minimizes incubation times, and reduces the need for extensive washes of the sperm cell pellet normally employed in traditional differential extraction procedures.

*** Collegiate Competition**

PowerPlex® Fusion: An Expanded Multiplex for New Global Standards

Danielle Brownell, Forensic Regional Account Manager, Promega Corporation

Abstract:

As DNA databases grow and international cooperation increases, a common set of markers is a prerequisite to facilitate data sharing and to help 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), and generates profiles compatible with databases based on either the proposed CODIS expanded core or European Standard Set (ESS) requirements. 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. The presentation will include an overview of the system as well as a review of the data generated during the developmental validation of the kit. The developmental validation demonstrated the quality and robustness of the PowerPlex® Fusion System across a number of variables following 2012 SWGDAM guidelines. Reproducible and high-quality results were compiled using data from twelve separate forensic and research laboratories. The results verify that the PowerPlex® Fusion System is acceptable for use by human identification laboratories.

Formula for success: how one lab addresses form and function when presenting DNA profiles for statistical analysis.

Cristina P. Somolinos, MLIS, D-ABC - DNA Laboratory, Office of Forensic Sciences, New Jersey State Police

Abstract:

Demonstration and discussion of merits of customized worksheets vs. using the PopStats module available in the CODIS Analyst Workbench. Overview of our solution: one-page, three-population Excel worksheets:

- Random match probability (RMP), with 2p calculations for single source profiles
- Llikelihood ratio (LR), for two-person mixtures
- Unrestricted (or "modified") random match probability (uRMP), for two-person mixtures
- Combined probability of inclusion (CPI).
- Paternity Trio - Combined Paternity Index & Probability of Paternity

Discuss experience with validation, comparison with PopStats. Changing/creating custom frequency databases in PopStats.

Customizing to your chemistry's ladder calls (variants, ladder real and virtual bins). Minimum allele frequencies. Comparison to accepted NDIS allele ranges.

Exporting results from GeneMapper IDX into summarized view in Excel for inclusion in the case file.

Northeastern Association of Forensic Scientists

Analyzing Mixtures in a DNA Profile

Alyssa Badgley and Chelsea Dodge - Indianapolis State Police Crime Laboratory

Abstract:

A two-person mixture is a common occurrence DNA analysts face when analyzing evidence submitted to a laboratory. However, sometimes a mixture appears to include more than two people. This project, completed by two interns at the Indiana State Police Laboratory, found that many times an analyst will estimate too low on how many people are actually included in a DNA mixture. A total of four hundred eighty-four profiles of unrelated individuals and fifteen loci were used to generate a certain number of combinations for a two, three, four, five, and six-person mixture. Not all combinations could be reported due to the factorial nature of determining the total number of possible combinations; however, the smallest sample size had 116,887 combinations (two-person mixture). The results were found under ideal conditions such as no allelic drop-out, no stutter, no artifacts, all alleles were above the stochastic threshold, and all mixtures were "perfect" with 1:1 or 1:1:1 etc. ratio which is not indicative of real casework in the laboratory but a best-case scenario. The goal of the project was to determine the minimum and maximum alleles observed for each profile, the average number of alleles observed per locus, and how many times a specific number of alleles is observed in a mixture. In addition, each locus was analyzed to determine how many combinations showed a certain number of alleles for each mixture. This information suggests certain loci may determine how many people are in a mixture more accurately than other loci. The presentation includes pie graphs and charts corresponding to the data collected for each mixture (two to six-person) analyzed. According to the results found, a two-person mixture is easily distinguishable when compared to other mixtures, a three-person mixture will show a maximum of five or six alleles about 90% of the time, a four-person mixture only indicates the fourth person about 40% of the time, a five-person mixture looks like a four-person mixture almost 80% of the time, and a six-person mixture excludes the fifth and sixth person about 70% of the time. Based on these results, in a best-case scenario an analyst has no strong supporting data to call anything more than a two-person mixture.

***Detection of Spermatozoa from Challenged Samples**

Allison Jacobs and Reena Roy, Ph.D - Pennsylvania State University, Forensic Science Program, Eberly College of Science

Abstract:

An integral aspect of a sexual assault case is to detect spermatozoa. However, it is often difficult to detect spermatozoa particularly when semen has been exposed to environmentally insulted conditions or the victim does not report the crime until several days later, thus resulting in having few spermatozoa in the stain extract. Limited amount of semen poses challenge when mixed with other body fluids containing numerous epithelial cells such as from the vaginal, rectal, or oral cavities. This is also true about bestiality cases where human semen may be mixed with semen from animals. The microscopic identification of spermatozoa by conventional means is time consuming and in challenged samples, it is often difficult to search for and identify them.

This research project has two objectives: the first to identify human spermatozoa from challenged samples and the second to compare the results of fluorescent staining of human spermatozoa with that of the

commonly used acidophilic and basophilic stains. Normally, in forensic laboratories Kernechtrot-Picroindigocarmine (KPIC) stain, commonly known as the Christmas Tree stain, is used to identify spermatozoa. The nucleic acid contained in the heads of the spermatozoa is stained red and the tail, green. A more recent technology from Independent Forensics known as SPERM HY-LITER™ contains reagents capable of staining the spermatozoa heads with a fluorescently labeled monoclonal antibody. This antibody is specific for an antigen situated on human spermatozoa heads. Since sophisticated fluorescent microscopy along with computer software is not available to all crime laboratories, this research was conducted with a microscope attached to a source of fluorescence. Instead of recommended slides and coverslips, ordinary laboratory glassware used for identification of KPIC stained spermatozoa were used with this fluorescent technique.

The detection of human spermatozoa from environmentally challenged samples using KPIC and fluorescence microscopy was performed by preparing several simulated evidence samples. Different concentrations of human semen containing spermatozoa were deposited on various substrates, including fabrics of different colors and compositions, and on soil, leaf, condoms etc. Fabric pieces containing semen were washed with and without detergent using a commercial washing machine. A measured amount of semen was also mixed with other body fluids such as blood and saliva from male and female donors. Each sample was extracted and each extract was divided into two slides. Extract deposited on one slide was stained with reagents in SPERM HY-LITER™ kit, while the other was stained with the KPIC stain. Slides stained with KPIC were examined with a regular microscope. Spermatozoa stained with reagents in the SPERM HY-LITER™ kit were identified with a regular microscope attached to a fluorescent source.

The results of this study indicate that the SPERM HY-LITER™ kit can detect human spermatozoa from a mixture of body fluids and is more sensitive than the standard KPIC stain. Spermatozoa heads were detected from almost all of the environmentally challenged samples using fluorescence staining. The results of the research indicated that fluorescent detection technique is more sensitive than the KPIC staining of spermatozoa, particularly when the evidence samples are challenged.

*** Collegiate Competition**

Development and validation of the Yfiler Plus™ PCR amplification kit, a new highly discriminating Y-STR Multiplex for Forensic Applications

S. Gopinath, Jill Muehling , C. Bormann Chung, J. Mulero, M. Danus

Abstract:

Y-chromosomal markers have proven useful in solving investigations where low levels of male DNA are present in a high female DNA background. An intrinsic limitation of Y-STRs compared with autosomal STRs is a reduced power of discrimination due to a lack of recombination throughout most of the Y-chromosome. Thus, in an effort to increase the power of discrimination we have developed a new 6-dye, 27-plex Y-STR system that includes the 17 markers from the AmpF�STR Yfiler kit plus 10 additional highly polymorphic Y-STR markers (DYS576, DYS627, DYS460, DYS518, DYS570, DYS449, DYS481, DYF387S1a/b and DYS533). These ten new loci include 7 rapidly mutating Y-STR loci which allow for improved discrimination of related individuals.

The new multiplex is a dual application assay designed to amplify DNA from extracted casework samples and database samples from storage cards and swab lysates via direct amplification. Compared to the previous Yfiler® Kit, the new multiplex shows improved performance in inhibited samples, faster time to results, admixed male and female samples at ratios >1:1000 and better differentiation in male:male mixture samples in high female DNA background. Additionally under optimized conditions, no reproducible cross-reactive products were obtained on bacteria and commonly encountered animal species. The haplotype diversity and discriminatory capacity calculations for several population groups will be presented, as well as father-son studies and validation studies demonstrating improved performance with challenging samples.

***NOCIt – A Computational Tool to Infer the Number of Contributors to a Forensic DNA Sample**

Harish Swaminathan[1], Catherine M. Grgicak[2], Muriel Medard[3], and Desmond S. Lun[1]
[1]Center for Computational and Integrative Biology, Rutgers University, Camden, NJ 08102, USA
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[3]Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Abstract:

A biological sample obtained from a crime scene might be a mixture containing DNA from two or more individuals. An assumption about the number of contributors to a sample is needed to compare the crime scene profile with that of a known if the Likelihood Ratio is the statistic utilized to convey the 'weight of the evidence'. Usually, the number of contributors to a question sample is unknown and is specified by the analyst based on the electropherogram obtained. This can be challenging in the case of complex, low template samples that often contain artifacts like dropout and stutter. We present NOCIt – a computational tool that calculates the probability distribution for the number of contributors to a DNA sample. Unlike existing methods to determine the number of contributors that operate only upon the number of peaks in the signal and/or the rarity of the alleles, NOCIt also uses the quantitative data in the signal, i.e. the heights of the peaks.

NOCIt was calibrated using single source samples amplified from various low level DNA amounts (0.007 – 0.25 ng) and using three different times of injection into the capillary for electrophoresis (5, 10 and 20s). The peak heights (peak height ratios in the case of stutter peaks) were modeled using the Gaussian distribution and appropriate parameters (mean and variance) were obtained from the calibration data. Dropout rates were also computed at each DNA amount.

NOCIt was run on 1, 2, 3, 4 and 5 person mixtures. The number of contributors with the highest probability was picked as the answer supported by NOCIt. In addition, the performance of NOCIt was compared with the Maximum Allele Count (MAC) and the Maximum Likelihood Estimator (MLE) methods. MAC uses the number of peaks in the signal at a locus to determine the minimum number of contributors while MLE uses the number of peaks as well as the qualitative data obtained, i.e. the allele frequencies. The performance of NOCIt was consistently better than MAC and MLE across all DNA amounts and all times of injection. Moreover, NOCIt had a high accuracy rate at the higher DNA amounts (>0.0625 ng) at all three injection times. Even in cases where NOCIt fails to pick the correct number of contributors, it can identify the region in which the number is most likely to lie. This could be useful in the case of complex and/or low template samples. Moreover, the software also provides the most likely set of alleles at each locus, which can be made use of while performing statistical calculations. NOCIt has been implemented using the Java programming language.

*** Collegiate Competition**

Improvements in Forensic STR data analysis with GeneMarker® HID and Next-generation sequence analysis of mtDNA with NextGENe® Software

Teresa Snyder-Leiby, Ph.D., SoftGenetics, LLC State College, PA

Abstract:

Short Tandem Repeat (STR) technology is constantly improving in sensitivity and power of exclusion. Sensitivity can be improved thanks to low copy number (LCN) techniques that are often able to provide full profiles from swabbed surfaces. The increased number of loci in new STR kits enhances the exclusionary power of the data and decreases the number of false positives from reference samples and familial searches. Positive familial searches of male profiles can be refined by Y-STR comparison. These improvements will be apparent in the first half of this presentation, during which results will be shown for several samples amplified with PowerPlex® Fusion and Globalfiler® . These results were generated using GeneMarker HID software, an accurate, rapid analysis tool with linked navigation and post-genotyping applications to aid forensic analysis and decrease backlogs. Later, we will summarize mtDNA analysis and discuss the advantages of using NGS technologies for forensics mtDNA applications.

Examination of Proposed Manufacturing Standards Using Low Template DNA

Danielle Brownell, Forensic Regional Account Manager, Promega Corporation

Abstract:

Forensic DNA laboratories rely on reagent and plastics manufacturers to supply high-quality products with minimal interference from contaminating DNA. With the increasing sensitivity of short tandem repeat (STR) amplification systems, levels of DNA that were previously undetected may now generate partial profiles. To address the concern of laboratories worldwide regarding the potential of low-level DNA contamination in consumables, accrediting bodies in the United Kingdom and Australia proposed guidelines PAS377 and ISO 18385, respectively, for minimizing the risk of human DNA contamination events during the manufacturing process.

The guidelines also propose both the acceptable limits for “contaminating DNA” as well as the methods for detecting the potential contaminant. The UK guideline recommends using only STR testing, while the Australian guideline allows both STR and quantitative polymerase chain reaction (qPCR) as suitable methods. This paper compares the sensitivity of qPCR to STR analysis and discusses the suitability of each method in the manufacturing process for the purpose of certifying a product as Forensic Grade.

To determine the sensitivity of STR analysis, we analyzed the sensitivity of its two major components: the capillary electrophoresis (CE) instrument and the STR reagents. To determine the sensitivity of the CE instrument, we amplified a high amount of DNA (500pg) to eliminate the stochastic effect of amplification of low template DNA amount. This ensured that any dropouts at low input amount are due to CE limitation and not PCR variability. We tested instrument sensitivity using default and enhanced conditions as recommended by the UK guideline: longer injection and lower peak calling threshold. Under enhanced conditions, the limit of detection (LOD) for the CE instrument is 0.5pg.

The UK guideline also allows for replicate analysis as performed in low copy number (LCN) analysis. DNA input titration followed by analysis under LCN analysis (repeat injection followed by consensus allele calling),

sensitivity down to 1pg can be achieved. With input DNA of 5-10pg, which is approximately equivalent to the amount of DNA in one cell, 30-60% of the alleles were called. This would not meet the criteria suggested by the proposed guidelines as being Forensic Grade.

In contrast to STR analysis, qPCR analysis is sensitive down to 0.25pg input DNA. In addition to increased sensitivity, qPCR analysis is more suitable for testing a higher number of samples: more cost effective and simpler data interpretation. Testing a large sample number is necessary for increased statistical confidence in a destructive test where a representative sample from each batch is tested and destroyed. Therefore, we propose that qPCR analysis is used for testing plastic consumables. For STR reagents, we propose using STR analysis as it will simultaneously test all components of the kit for presence of contaminating DNA. While this presentation discusses the LOD for the test methods, the limit that is acceptable to the forensic laboratories still needs to be determined.

This presentation will impact the Forensic community by allowing the community to understand testing constraints in manufacturing processes and why multiple analysis methods are needed for Forensic Grade certification.

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Criminalistics Abstracts

In Pursuit of a Forensic Document Scanner - Combining Spectroscopy and Document Analysis on a Budget

Dr. John Allison, Kelly Wisnewski - Department of Chemistry, The College of New Jersey, P.O. Box 7718, Ewing NJ 08628

Abstract:

Perhaps the most substantial investigation into a document was that of the “Archimedes Palimpsest”, a thirteenth century prayer book made from an older document, by erasing the text and reusing the parchment. The original text, which had been written by Archimedes, was “spectroscopically recovered”. The project, which took almost a decade, basically used a collection of images collected using different wavelengths of light, from the UV through the IR. Researchers then wrote software to process the images, extracting the almost-invisible text, and suppressing the newer, darker writing. This led to the idea that, if one could use a scanner that irradiated a document at specific wavelengths, with a very narrow bandwidth, again from the UV through the IR, then a variety of questions in the area of document analysis could be approached (such as studying obliterations, investigating the use of multiple pen inks in a written document, etc.). This presentation discusses instruments and methods that allow such work to be done, including their strengths and weaknesses. Also presented is the initial concept of a forensic document scanner, and early investigations into the approach, focusing first on distinguishing between pen inks on paper. The challenge is a substantial one – the light source must be variable, over a broad range, capable of generating reasonably intense light with a narrow bandwidth. The light must be manipulated to illuminate a document. Images must be collected with a black and white “camera” that does not contain the filters that normal cameras have (to block IR and UV light). Finally, powerful software is required for image manipulation. These issues will be discussed, and results from a prototype presented.

Barely a Trace of Trace Evidence Sections: How to Keep Trace Evidence Relevant In Today's Criminal Justice System

Ted Schwartz, Daniel Rothenberg and Brandi Clark - Westchester County Forensic Laboratory

Abstract:

There is a disturbance lately in forensic laboratories. With the recent advances and successes in Forensic Biology, many laboratories are scaling back and even –eliminating the Trace Evidence section. There is no denying that many more cases have been solved in recent years with DNA than with Trace Evidence. As more cases are solved by DNA, more investment in the section is made. An obvious funding disparity is present.Trace Evidence is losing it’s appeal to politicians, lawyers and even many in the forensic science field; fostering the perception that there is much more “bang for the buck” with Forensic Biology. Within a typical local laboratory in “today’s economy”, grant money may be the main source of funding. So what section will remain and what will be cut?

It is easy for trace evidence examiners to sit back and feel badly about this trend. After all, many examiners have been moved into other sections. Some have joined their laboratory's DNA section as "set-up" technicians. Others have even lost their jobs altogether. Is there anything that can be done to stop and /or reverse this trend?

The truth is that it is up to us, the trace evidence examiners, to make people in the Criminal Justice System realize the importance of our discipline. We as trace evidence examiners, are still able to see things differently. We know that not every case hinges on DNA. The percentage of cases solved may be smaller than DNA, but that makes it no less valuable. Trace Evidence contributes to homicides, sexual assaults and every other crime worked on by DNA. Early forensic laboratories had a term "Criminalistics" which often referred to the combined work of Biology and Trace Evidence. Let's remind the field of that term. Often our cases involve interesting evidence with visual appeal and exciting results. When a TV show or class wants an "interesting case", which is the section that often provides that case?

In this paper, several ideas will be presented that might just help local laboratories reverse this trend. Making pamphlets to have available to officers in the evidence receiving area; giving lectures at local crime scene training seminars; utilizing databases. These and other approaches will be discussed.

An Assessment of Critical Angle Impacts for the 9 mm Bullet

Peter Diaczuk - Pedico Research Institute

Abstract:

The determination of the angle at which a bullet will successfully ricochet is a valuable asset if a shooting investigation involves indirect fire. There will be occasions when the bullet fails to ricochet, and occasions when the bullet does ricochet. Knowledge of bullet behavior with some common substrates will provide useful information when assessing scenes where bullets have interacted with intermediate targets.

Contrary to the definition of critical angle when used in the context of light rays, lenses and prisms, the term "critical angle" when referring to bullet impacts is defined in the literature as the angle at which a ricochet no longer takes place.

Broadly defined for the purposes of this study, substrates can be divided into two categories, yielding and non-yielding. For a yielding substrate, which is relatively soft and can be readily deformed, the definition of critical angle is easily applied- the bullet either penetrates or perforates the material. Two common examples of yielding substrates are gypsum drywall and sheet metal as used in automobile bodies. For the much harder and durable category of non-yielding substrates the definition of critical angle is not as clear-cut. Technically, even if the bullet breaks up on impact into fragments, and those fragments rebound off of the substrate, they have ricocheted. Examples of two common non-yielding substrates are marble and steel plate.

Using some common substrates this research was conducted to determine the critical angles of full metal jacket 9 mm Luger bullets in an effort to better understand the dynamics of substrate failure and bullet failure as applied to shooting reconstruction.

A Survey of the Ethical Environment of the Practice of Criminalistics

Edward G. Bernstine, Ph.D., Department of Biology, Bay Path College and Justin H. Dion, J.D., Department of Legal Studies, Bay Path College

Abstract:

Criminalistics is practiced at the complex intersection of science, law and criminal justice.

Criminalistics is science and, as such, it should be conducted according to accepted scientific standards promulgated by the scientific community. Such standards are normative statements that have the force of ethical mandates to scientists. Examples are the responsibility to conduct thorough scientific investigations before formally reporting results and, when reporting results, to make inferences that are best supported by laboratory results while nevertheless articulating other explanations that remain viable possibilities.

Trial law is an adversarial endeavor in which each side is obligated to try to prevail: prosecutors are obliged to try to get convictions and defense attorneys are obliged to try to get acquittals. Aside from many codified prescriptions and proscriptions, criminal trial attorneys are ethically bound to do their best to win their cases. Both often use science as a means to reach this end.

The criminal justice system itself functions within the ethical structure of our society and its various levels of government. The outcomes of criminal trials, representing efforts of science and law, should therefore be consistent with this larger ethical structure, one of whose components is, simply put, fairness.

In the first half of this brief presentation, we will discuss some of the ethical requirements of both professions and will then use the remaining time to foster an open discussion of our analysis and its implications.

***Measurement of Error in Traditional Methods of Angle of Impact Calculation Used in Bullet Trajectory Determination**

Shannon Wenner[1], Peter Diaczuk[2], Lawrence Quarino, Ph.D.[1] 1Cedar Crest College, 2Pedico Research Institute

Abstract:

The measurement of the angle of impact of a bullet striking a surface is necessary for proper bullet trajectory determination often needed in crime scene reconstruction. Traditionally, this has been done with methods using trajectory rods, string, and lasers. Appropriate diameter trajectory rods are placed in bullet holes and the directionality of the rod serves as the hypotenuse in a right triangle made with the surface containing the bullet hole. Similarly, string running through the center of both sides of the bullet hole serves the same function. Lasers can be used if the light is allowed to pass through the bullet hole and projected onto another surface. This projected point serves as the intersection between the hypotenuse and the side adjacent to the bullet hole surface in the right triangle. In all three cases, basic trigonometry can be used to calculate the angle of impact. Alternatively, a protractor can simply be used in tandem usually with a trajectory rod to measure the angle of impact. In this study, the four methods were applied to bullet holes which were fired into drywall at known impact angles of 20°, 30°, and 45°. The three different angles were made with three

different firearms and ammunition (for the 20° bullet holes a Colt Government Model firearm was used with 45 ACP caliber ammunition; for the 30° bullet holes a Ruger PC-9 firearm was used with 9mm Luger ammunition; for the 45° bullet holes a Colt AR-15 firearm was used 5.56 mm ammunition). The angle of impact of each bullet hole was performed five times with each method (N=30 for 20°, N=45 for 30°, and N=25 for 45°). Results showed that for the trajectory rod method, the trajectory rods with protractor method, and the laser method, mean values were within 2° of the actual value for all three angles. The type of ammunition used in this study did not affect the results. Only the string method produced discrepant results. Analysis of variance testing did show significant differences between the four methods and between the three methods with concordant results at the 95% confidence interval. Despite this, the practical effect is negligible between the three seemingly reliable methods.

*** Collegiate Competition**

***Investigating the Molecules of "Death"**

Rachel Bower, MPS Candidate at The Pennsylvania State University and Dr. Dan Sykes, Senior Lecturer and Director, Analytical Instructional Laboratories at The Pennsylvania State University

Abstract:

Decomposition is a very complex process that is not very well understood. Several studies have investigated the accumulation of volatile organic compounds (VOCs) produced during the early stages of human decomposition. However, our knowledge of the chemistry of decomposition is limited because of poor reproducibility and comparability within and between published studies and the difficulty in obtaining human corpses for decomposition studies. Several studies have shown that pig (Porcus) carcasses develop similar decomposition profiles to those of human corpses. Therefore, a comprehensive investigation (with larger and consistent sample sizes) of VOC profiles using pig (Porcus) carcasses is in progress which will provide important information about the unknown chemical composition of death.

A huge concern of the studies on decomposition that are published thus far, is that the sample sizes are less than desirable for valid scientific claims. However, these studies are still extremely important and provide the scientific community with important information. The main purpose of this study is to work towards establishing a more complete profile of the temporal evolution of volatile organic compounds (VOCs) released during the decomposition process by utilizing a larger sample size. Specifically, this study focuses on the most prevalent VOCs that are detected during each stage of decomposition. VOCs will be collected, identified and quantified from decaying pig carcasses using passive headspace collection via solid phase microextraction (SPME) fibers. The VOCs will then be analyzed and quantified using a gas chromatograph-mass spectrometer (GC-MS) instrument. As stated previously, pigs will be used as models for humans and the pigs will be euthanized via captive bolt as to ensure no chemical interferences. This study is broken into two phases; an indoor phase and an outdoor phase. Decomposition in an indoor enclosure will eliminate many of the uncontrolled factors that affect decomposition. This will provide information as to whether a consistent VOC profile is obtainable. Decomposition outdoors will take into account insect activity and the various weather factors such as rainfall and temperature.

Unfortunately, a major area and one of the reasons for forensic science even existing is criminal activity in the form of murder. A problem within this crime is that many of the perpetrator's will try to hide their acts by concealing the victims. When the bodies are not readily evident to the investigators, they are considered clandestine burials and decomposition can become a factor. These burials are a twofold problem; it impedes the investigation and

thus creates a longer timeframe that the friends and families of the victims must wait for answers and closure. Working towards establishing a more complete profile of the temporal evolution of VOCs released during the decomposition process will impact and improve models which predict interval since death, insect succession, and possibly, lead to alternate methods for identifying clandestine burial sites.
*** Collegiate Competition**

***The Prevalence of Intact Sperm Cells in Incidents of Sexual Assault: A Case Study**

Kaitlin Rogers and Amy Brodeur - Boston University School of Medicine, Erica Neu and Kathrynne St. Pierre - Boston Police Department Crime Laboratory Unit

Abstract:

The literature on sexual assault cases that present time frames for detection of various seminal components commonly tested for in forensic laboratories is limited in quantity and scope. Determining an accurate time since intercourse (TSI) interval based on an extensive review of forensic casework would provide investigators with a tool for estimating the time elapsed between the occurrence of a sexual assault and the collection of a Sexual Assault Evidence Collection Kit (SAECK). The presence and percentage of intact spermatozoa is an important finding in establishing TSI, which could be vital information in certain cases. This study seeks to use forensic casework to demonstrate that a percentage of sperm tails is lost during the preparation of Kernechtrot Picroindigocarmine (KPIC) stained microscope slides from SAECK swabs through a direct comparison with smear slides prepared by medical personnel from the same intimate swabs. Additionally, this study seeks to present TSI intervals for detection of acid phosphatase, sperm heads, intact spermatozoa, and prostate specific antigen based on a thorough review of cases examined in the last five to ten years at the Boston Police Department Crime Laboratory Unit. Results demonstrate that the percentage of intact sperm cells observed is lower in extract-prepared slides than in corresponding smear slides. Results also show that the amount of time elapsed between the alleged assault and the collection of the SAECK as well as the victim's intermediate activities must be considered when estimating TSI in forensic cases.

*** Collegiate Competition**

The Impact of Temperature and Humidity on Overlapping Wipe and Drip Bloodstain Patterns

Corey W. Scott and Virginia Maxwell, D.Phil. – University of New Haven, West Haven, CT

Abstract: The purpose of this study was to observe the physical characteristics of overlapping bloodstains when exposed to increased humidity and decreased temperature. Wipe and drip patterns were created with the use of 20 and 50 microliter samples of fetal pig blood on multiple surfaces commonly found at a crime scene to include ceramic tile, cardboard, linoleum flooring, and carpet. It is possible to determine the order at which a droplet or wipe patterns occurred based upon the presence of a substrate or red ringed image around the drip pattern; however, when exposed to increased humidity, there was a significant difference compared to the control samples.

Beatrice Six: A rape and murder case in a small town in Nebraska

Reena Roy, Ph.D. - Associate Professor, Penn State University Forensic Science Program, Eberly College of Science, 107 Whitmore Lab, University Park, PA 16802

Abstract:

In the early morning hours of Feb. 5, 1985, a 68-year old woman by the name of Helen Wilson was brutally raped and murdered in a small town called Beatrice, Nebraska. Her small apartment was not ransacked, and a large amount of cash was found in her purse, which appeared untouched by the perpetrator.

After four years of investigation, in 1989, three women and two men confessed to the crime to avoid death sentences. They claimed that five of them and a sixth man broke into Wilson's apartment to rob her, but the attempted robbery turned into a rape and homicide.

The sixth individual, Joseph White, insisted he was innocent and refused to plead guilty. He was charged, convicted in a jury trial, and sentenced to life. He lost his appeal, but maintained his innocence and kept trying to have DNA testing done on the evidence.

Finally, in 2008, all six individuals were excluded after testing of blood and semen at the crime scene. The six individuals became the first people in Nebraska to be exonerated by DNA testing under the DNA Testing Act passed by the Nebraska Legislature in 2001.

The crime was committed by one man, Bruce Allen Smith, who grew up in Beatrice, returned to town days before the slaying and then quickly went back to Oklahoma. Instead of sending his samples to Nebraska, his known blood and saliva samples were sent to a lab in Oklahoma City and he was excluded.

From Hook Up to Shook Up: The Darker Side of NYC Nightlife

Meredith Rosenberg - NYC Office of Chief Medical Examiner, Department of Forensic Biology

Abstract:

In New York City, a population of 8.2 million, there were 142, 760 property crimes reported in 2012. According to the FBI's Uniform Crime Reporting (UCR) Program, property crimes include the offenses of burglary, larceny-theft, motor vehicle theft, and arson. The object of the theft-type offenses is the taking of money or property, but there is no force or threat of force against the victims. The New York City Office of Chief Medical Examiner's (OCME) Department of Forensic Biology performs DNA testing on property crimes but also includes "robbery" cases. Robbery cases are when force or the threat of force was used during the commission of the crime.

A robbery case pattern that has become evident within the Property Crimes group of the OCME is comprised of cases where the complainants meet consensual sexual partners through the internet, bars, clubs, and other social meeting spots. The complainants then bring the perpetrators back to their residence. After consensual sex (which is either stated or implied in the police report), the complainant is either robbed or robbed *and* physically assaulted.

The presentation will describe what types of items have been stolen from the complainants as well as what evidence was collected from the individual crime scenes. It will exemplify that the cases associated with this pattern are not deemed sexual assaults (because the sexual act is consensual), but are deemed *larceny* cases. No sexual assault kits were collected based on a charge of rape for any of the cases that will be presented. All the evidence that has been collected is based on the property crime and/or the assault aspect for all these cases. The investigative leads developed from the DNA results will also be presented.

***An Analysis of Metal Content in Various Brands of Cigarette Ash by Atomic Absorption Spectroscopy**

Kaitlin Hafer, B.S. - Cedar Crest College, 100 College Drive, Allentown, PA 18104 and Lindsey A. Welch, Ph.D.

Abstract:

Among the literature investigating the metal content present in cigarettes, most focus on the negative effects towards the environment and health. Very little work exists to benefit the forensic science field. The purpose of this study is to investigate the ability to differentiate ash from American cigarette brands based on their metal content. The ability to link cigarette ash to a specific brand could assist analysis of trace evidence in a case. In this study, an Atomic Absorption Spectrophotometer (AAS) was used to develop a less expensive method of analysis compared to Inductively Coupled Plasma – Mass Spectrometry. To obtain cigarette ash, a "smoking apparatus" including a Swagelok valve and a vacuum pump was built to assist in smoking each cigarette sample. Cigarettes from Camel Blue, Marlboro Medium Red, Newport and Parliament cigarette packs were smoked for ash collection. Ash samples were digested in 2mL of concentrated nitric acid followed by the addition of 2mL of concentrated hydrochloric acid. Upon filtration, the product was diluted to 25mL with deionized water. Trace metal grade standards were used to generate calibration curves with concentrations in the parts per million (ppm) range. A Buck Scientific ACCUSYS 211 AAS was used for analysis. The metal content for each ash sample was then determined. Four metals, calcium, potassium, magnesium and zinc, were analyzed for each sample. The metal concentrations for each brand were measured and compared using discriminant analysis through XLSTAT 2013. Based on the metal content of cigarette ash, different brands are distinguishable from one another. Using this method, the extent of discrimination between brands was determined.

*** Collegiate Competition**

The Chef in a Bag

Detective Dan Trompetta - Major Case Unit, Danbury Connecticut Police Department

Abstract:

On 24 April 2004, at about 3 PM a young boy participating in an "Earth Day" clean- up at the end of a cul-de-sac just off Interstate 684 in Bedford, New York discovered a black plastic bag containing a human leg. Police were notified and additional bags were located containing human body parts, including a head. During the autopsy, it was learned that these were the remains of Zef Vulevic, a prominent chef in the CT/NY area. He had been shot twice in the back of the head & cut into seven pieces. Vulevic had been reported missing by his nephew and business partner sixteen days earlier in Connecticut.

Over the next four years the homicide investigation was ongoing. This investigation involved: the Danbury Police, Danbury State's Attorney's Office, Westchester County District Attorney's Office, Westchester County Medical Examiner, The Forensic Laboratories of Westchester County as well as the State of Connecticut, U.S. Marshal Service, Federal Bureau of Investigation (New York and Mexico), and the U.S. Attorney's Office (SDNY). Forensic experts were consulted, namely: William L. Krinsky, MD, Ph.D. Division Entomology at the Peabody Museum of Natural History at Yale University, Forensic Pathologist Dr. Michael M. Baden, Director of the Medicolegal Investigations Unit for the New York State Police, and Steven A. Symes Ph.D., DABFA Professor of Applied Forensic Sciences and Anthropology at Mercyhurst College.

Organized crime figures were also explored & interviewed. On 08 March 2008 an arrest warrant was issued at the Danbury Superior Court in Connecticut. Following a six week trial a Guilty verdict was returned by a 12 member jury to the sole count of Murder.

Linked by Strands of DNA – The Long Trail from Victims to Verdict

Mary M. Eustace, Westchester County Forensic Laboratory

In 1986, a young girl on her way home from the grocery store was found raped and suffocated on a path near her home. In 1987, the frozen decomposed body of a woman, who was last seen at a New Year's Eve party, was found in a deserted wooded area. In 1990, a woman on her way to work in the early morning hours was pulled from a walkway, raped, and shot in the head.

These tree seemingly unconnected homicide cases, investigated by three separate police departments, were ultimately associated through the cooperative efforts of criminal investigators and forensic scientists.

Little did the three victims or their families know that over the next 15 years their cases would be interwoven through strands of DNA.

This presentation will discuss the elements of each crime, the varied technologies used to analyze the evidence, and the critical decisions that were made during the investigation.

***Differentiation of Human Hair by Color and Diameter Using Digital Imaging and Discriminant Analysis**

Morgan Mills and Larry Quarino, Cedar Crest College, Allentown, PA

Abstract:

Since hairs are continually shed and are easily removed from an individual, they are a common source of transfer evidence in most types of criminal assaults. Although hair can have important evidentiary value in indicating that contact occurred between individuals, or between an individual and a crime scene, the associative value of hair is often questioned when comparative analysis is based on microscopic comparison, due largely to the subjective nature of the examination.

To assess the viability of this method, ten hairs from each of eight participants, all with naturally colored brown hair were mounted on microscope slides with Meltmount® (refractive index of 1.539), and examined on an Olympus BX53® polarizing light microscope under Kohler illumination. Digital images were viewed with an Olympus DP72® camera under 400x after white balance correction. Using Olympus cellSens Entry® software,

the diameter of the hair was measured at the following distance intervals from the base of the root: 500-600um, 600-700um, 700-800um, 800-900um, 900-1000um, 1000-1100um, 1100-1200um, 1200-1300um, 1300-1400um, and 1400-1500um. A minimum distance of 500um was selected because previous validation indicated that color measurements begin to show consistency at this point. At each distance interval, a quantitative value for red, green, and blue was determined by the software at three points along where the hair diameter was measured. In an additive color model, various combinations of red, green, and blue are used to describe a wide range of colors. The software uses an RGB color model to define each pixel on an image by its principal color components of red, green, and blue. The intensity of each component is provided as an integer ranging from 0-255. Lower values represent less intensity and thus a darker shade of that color component. Therefore, the color gray is described by equal intensities of all three color components relative to each other, while the particular shade of gray is dependent upon how high or low these intensity values are. The intensity of the source was preset to ensure that illumination remained constant prior to the start of hair examination each day during the study. In addition, consistency of the illumination was verified by testing a standard hair to ensure that color values did not fluctuate from day to day.
All diameter and color values for each hair were compared using discriminant analysis (XLSTAT®). Results showed that variation of hairs from each individual was far less than hairs between the eight individuals. Two-dimensional analysis showed that hairs from the same individual tended to cluster, easily distinguishing the eight individuals. Pairwise comparisons between individuals resulted in p-values significantly less than 0.05 indicating significant differences. These results thus show the potential of this method to offer subjective analysis of microscopic hair comparison.

*** Collegiate Competition**

***Raman Microspectroscopic Chemical Mapping, a Novel Approach for Gunshot Residue Detection on Adhesive Tape**

Justin Bueno and Igor K. Lednev - *Department of Chemistry, University at Albany, SUNY, 1400 Washington Avenue, Albany, NY 12222, USA*

Abstract:

Gunshot residue (GSR) consists of the unburnt and partially burnt byproducts that are expelled during a firearm discharge. Detection of GSR at a crime scene provides critical information useful for crime scene reconstruction. Recovery and detection of GSR from a person of interest indicates the person had some degree of involvement in the shooting incident. “Tape lifting” or “tape collection” is one of the most common and inexpensive methods used for the recovery of GSR particles. Tape lifting is the application and pressing of double-sided adhesive tape to a surface containing GSR. GSR particles are collected on one side of the tape, while the other side of the tape is mounted to an appropriate stage for analytical analysis. Unfortunately, using current GSR detection methodology (scanning electron microscopy combined with energy dispersive spectroscopy, SEM/EDS), several drawbacks may be encountered when attempting to locate GSR particles adhered to tape substrates.

A novel approach utilizing automated Raman microspectroscopic mapping for gunshot residue (GSR) detection was investigated. Raman analyses of the tape substrates were performed in ambient conditions, without the need of additional sample preparation. Double-sided adhesive tape (3M brand); organic and inorganic GSR (OGSR and IGSR) particles, were spectroscopically characterized before GSR collection. These samples were scrutinized to develop the Raman spectroscopic training set for each analyte. After tape lifting for GSR collection, automated Raman mapping was used to rapidly collect spectra over areas of the tape substrate populated by GSR particles. Raman spectra collected from the maps were considered as unknown, and compared against the training sets via

partial least square discriminant analysis (PLSDA), to determine if GSR was present. Results of the classification analysis (PLSDA) indicated the successful identification (with high confidence) of GSR particles with varying morphologies. Chemical maps were generated for visual detection of GSR with high spatial resolution. Preliminary results indicate the method can be applied to the detection to as little as one GSR particle. External validation of the method was performed on a different Raman microscope as compared to the training set experiments, indicating that the method is independent of specific Raman instrumentation or collection software. We report a preliminary limit of GSR detection (LOD) for particles with an approximate diameter of 3.4 m or greater, which is determined by the objective of the microscope.

*** Collegiate Competition**

Northeastern Association of Forensic Scientists

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