Applied Biosystems Product Update

CABALLERO, Catherine
Applied Biosystems, 850 Lincoln Centre Drive, MS 404, Foster City, CA 94404

Part 1: Developmental Validation of the AmpFLSTR® MiniFiler™ PCR Amplification Kit: a 9-plex miniSTR Assay for the Analysis of Compromised DNA Samples

Forensic DNA typing is facilitated by the employment of highly polymorphic STRs. Despite their relative small size (100-400 bp), DNA degradation due to environmental exposure could result in a lack of sufficient intact target fragments to generate a complete genetic profile. The problem is magnified when large multiplex STR reactions are used due to the wide fragment size range of the amplified PCR products e.g. the largest STR loci fall below the detection limit due to preferential amplification of the smaller loci.

In recent years, successful recovery of information from degraded DNA samples have been accomplished through reduction of the size of the STR PCR products by moving primers in as close as possible to the STR repeat region. In an effort to increase the amount of information derived from compromised DNA samples we have redesigned as miniSTRs the largest eight loci in the AmpFLSTR® Identifiler™ PCR Amplification Kit (D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO, FGA). Five of these loci (D16S539, D21S11, D2S1338, D18S51, and FGA) also represent five of the largest loci in the AmpFLSTR® SGM Plus® kit. Size reduction of the STR amplicons ranged from 33 to 208 bp. This highly informative 9-locus multiplex, which includes the sex determining locus Amelogenin, employees a 5-dye labeling technology and mobility modifiers to enable simultaneous CE separation of the DNA fragments. In this presentation, results from a developmental validation study of the AmpFLSTR® MiniFiler™ PCR Amplification Kit will be described.

Part 2: Streamlining the Validation of New Forensic DNA Technologies

As the demand for processing DNA evidence has continued to grow, so has the development of new technologies for DNA analysis. These factors can make it difficult for a crime laboratory to strike a balance between successful case workload management and the evaluation and implementation of new technologies. Laboratory Accreditation and Forensic DNA Analyst education require careful assessment and thorough validation studies to provide confidence in the DNA results, ensuring the generation of robust, reliable and reproducible data.

There are a variety of challenges the Forensic DNA laboratory faces when implementing a new methodology. A common challenge identified by laboratories is a lack of resources available for validation. Laboratories also point to the existence of diverse opinions with respect to validation protocols, sample numbers and definition of appropriate and effective experiments as notable challenges. These variables have been shown to contribute to extensive validation studies that include unnecessary or excessive tests without the benefit of additional confidence. In addition, data management and analysis are cumbersome processes that are often manual operations or utilize a series of tools which analysts have developed on their own.

This presentation will introduce attendees to time-saving tools and services developed by Applied Biosystems to significantly streamline the validation of new forensic DNA

technologies. First, VALID™ is a software program designed to help support, simplify and standardize validation studies while meeting SWGDAM/DAB recommendations. This is accomplished by incorporating the following functionality:

- Easy to use software program with a simple graphical user interface
- Experimental design tools and recommendations
- Integration of all portions of validation and workflow processes
- Calculation and data analysis tools
- Project and documentation management—including final report capabilities

Second, Applied Biosystems has created a Validation Support Services program, which provides the resources, manpower and deliverables to complete validation efficiently and effectively. In partnership with the client laboratory, and under the direction of the laboratory director, technical leader and quality assurance manager, Applied Biosystems executes the necessary validation experiments, including data analysis and reporting, to get instruments and chemistries on-line as quickly as possible while meeting all SWGDAM/DAB auditing and accreditation standards.

Part 3: Quantifiler® Kit and Allelic Ladder Updates

As the Quantifiler® Human DNA Quantification kit assays are increasingly adopted by human identification laboratories there have been requests for more information regarding the extent of variability in the assays. This presentation will discuss some of the factors that may contribute to variability within a single quantification method, some observed differences between various quantification methods and studies performed at Applied Biosystems to assess tube-to-tube and lot-to-lot variation in the Quantifiler assay. The manufacturing quality control procedures and expected range of variation in the Quantifiler DNA standard will be discussed with the aim of providing guidance to Quantifiler kit users for achieving optimal results. The presentation will also discuss results which may be obtained for non-template controls and emphasize procedures to minimize the detection of positive results for extraction blank and negative control samples. Finally, an overview of upcoming changes to the manufacturing procedures for the AmpFLSTR® kit allelic ladders will be provided with a summary of validation studies conducted to verify allelic ladder performance.

A Partnership Between Crime Laboratory Directors in the Southwest and Texas Tech University Health Sciences Center, Institute for Forensic Science
SPERRY, Kathy, and James M. Childers

Texas Tech University Health Sciences Center Institute for Forensic Science would like to invite crime laboratory directors in the Southwestern United States to be part of a proposed coalition to provide insight into educational and research needs within the fields of forensics and law enforcement.

Historically, there has not been a cohesive collaboration between practitioners and academic research and higher education. Texas Tech University Health Sciences Center Institute for Forensic Sciences is going to be one of the few universities that transform this historical phenomena.

One of the primary objectives of the Institute is to establish a dialogue that leads to a communication network of practitioners and the Institute. The Institute in conjunction with the Texas Tech University Health Sciences Center, Texas Tech academic campus,

and the Texas Tech School of Law are in the process of establishing a Master's degree in forensic science. The development of a graduate degree has included input from numerous crime laboratory directors and other forensic professionals. The degree program has been designed to meet the challenges facing the criminal justice system. A primary focus of the degree program will be directed towards preparing graduates with the requisite knowledge and the necessary skills to enter the forensic field.

In Situ Identification of Nickel Titanate and Chrome Titanate in Automotive Paints Using Extended Range FT-IR Spectroscopy (4000-220 cm^{-1}) and XRF Spectrometry

SUZUKI, Edward M.
Washington State Crime Laboratory

The identification, analysis, and occurrence in U.S. automobile original finishes (1974-1989) of Nickel Titanate and Chrome Titanate are described in this presentation. These two inorganic pigments have lemon yellow and golden yellow-orange hues, respectively.

The titanate pigments are based on the rutile (titanium dioxide) structure and there are only minor differences between the infrared absorptions of rutile and the titanates. Titanate pigment absorptions in paint spectra can thus be easily mistaken for those of rutile. Each of the titanates, however, contains two elements in addition to titanium that can serve to distinguish them using elemental analyses. Extended range FT-IR (4000-220 cm^{-1}) and XRF instruments were thus used in combination for the *in situ* analysis of the titanates.

In addition to titanium, nickel, and antimony, the three main detectable elements comprising Nickel Titanate, all of the commercial products of this pigment that were examined by XRF (using a tin secondary target) contained impurities of zirconium, niobium, and usually lead. These elements were also detected in most of the paints in which Nickel Titanate was identified, as well as in the Chrome Titanate pigments and paints. The relative levels of these elements vary, particularly the zirconium to niobium ratio, and this can serve to distinguish further paints containing a specific titanate pigment. These impurities arise primarily from the ores that are used to produce anatase, which in turn is used to produce the titanates. Additional zirconium may result from degradation of the dispersion beads that are used in the manufacture of the paint, if zirconium oxide beads are used.

Nickel Titanate is a relatively common pigment that was identified in nearly three dozen U.S. automobile yellow nonmetallic monocoats (1974 to 1989) from the Reference Collection of Automotive Paints (Collaborative Testing Services). Chrome Titanate appears to have been used in only a few yellow and orange nonmetallic monocoats. The use of the titanate pigments likely increased after this time period as they were replacements for lead chromate pigments, which were last used in a U.S. automobile original finish in the early 1990s. Titanates likely also become more common after 1989 because of the increasing prevalence of basecoat/clearcoat finishes. Heavy pigment loads are required with the titanates to achieve the vivid colors typical of many automotive finishes, and this makes it difficult to achieve a high gloss finish in the monocoat. This is not a problem with the basecoat/clearcoat finish, however.

Correlation of Physical Appearance of Hair Roots to Success Rate of Nuclear DNA Analysis

BANKS, Rhonda

Oregon State Police Forensic Laboratory

The ability to visually assess the potential success of nuclear DNA analysis of a hair root would be a valuable tool in making decisions regarding the consumption of hair evidence and its use for nuclear versus mitochondrial DNA analysis. This survey presents a compilation of data gathered from DNA analysis of hair roots in an attempt to determine if a correlation can be drawn between the physical appearance of a hair root and the ability to obtain a nuclear DNA profile.

Comparison of Five Forensic DNA Extraction Methods

KUPFERSCHMID, Timothy D., and Jonelle Thompson
Sorenson Forensics, 2495 S. West Temple, Salt Lake City, UT 84115

Many different extraction protocols are being used in the forensic community. One critical factor in choosing an extraction procedure is the ability to minimize the amount of inhibition present in an extract. Organic extractions are well known for removing a majority of the inhibitors. However, an organic extraction is a long, manual process that is not automatic.

Four different extractions were chosen, DNAIQ (Promega), ChargeSwitch (Invitrogen), Qiagen MicroPrep (Qiagen), and ForensicGem (ZyGem) in an effort to find an extraction procedure capable of being automated. Each extraction method had to have the ability of organic extractions to reduce the amount of inhibitors, while still obtaining a suitable quantity of DNA for STR analysis. To evaluate the efficiency of the procedures, both quantity and quality of DNA were compared.

Initially, two magnetic bead systems, DNAIQ and ChargeSwitch, were run following the manufacturers' protocol. Different fabric types with a variety of dilutions of blood were extracted. The quantitation results showed some evidence of inhibition. Modifications were made to each extraction protocol in an effort to optimize the extraction method. The second round of extractions was performed with a subset of the samples. The results showed reduction in inhibition, while increasing the amount of DNA isolated.

Subsequently, all five extraction methods were evaluated using challenging fabrics with blood, touched items, buccal swabs, hair, and cigarette butts.

This presentation will discuss which extraction was chosen for use in our laboratory. The protocol was chosen based on the quality of data compared to the organic extraction method currently being used in our laboratory.

Differential of Hair Dye Using Forensic Laboratory Instrumentation

CRAGO-STASICHA, Devin, and Stacey Walker
Texas DPS

The purpose of this study was to determine if different hair dyes that exhibited similar coloration could be differentiated using instruments in the forensic laboratory. To keep this introductory study manageable, only 10 commercially available hair dyes (six red and four black) were used. Fifteen people donated hair standards and each hair standard was dyed with the different brands of hair dye. The hair comparisons were performed within each group to eliminate varying hair characteristics as a factor for discrimination. Analysis of these hairs was conducted with the following instruments:

the comparison microscope, the Fourier Transform Infrared Spectrometer, Thin-Layer Chromatography, Pyrolysis Gas Chromatography/Mass Spectrometry, and the Microspectrometer. As was expected, the comparison microscope proved to be the most discriminating tool in distinguishing between different hair dyes. However, there were a few instances where the dyes were close enough in color that they appeared microscopically similar or inconclusive. For those hairs, the Microspectrometer proved to be reliable in distinguishing between two different dyes of similar coloration. The remaining instrumentation did not yield any useful results.

A New Type of GC/IR for Forensic Drug Analysis

KEARNEY, Tom, and Bob Shipman
Spectra Analysis, 257 Simarano Dr., Marlborough, MA 01752

The typical forensic laboratory has a heavy drug caseload and encounters drug exhibits, which often contain mixtures of compounds in addition to any regulated component. To meet analytical requirements and caseload demands, the forensic laboratory relies upon GC/MS to perform routine drug analysis by employing a technique that allows automated sampling, separation, and subsequent structural identification. Mass spectrometry does have limitations with some drugs yielding minimal mass spectra or similar spectra between compounds.

Infrared spectroscopy is also used for forensic drug analysis, is useful for the identification of compounds with similar mass spectra, and can differentiate diastereomers (pseudoephedrine/ephedrine) which cannot typically be identified using MS. The routine application of IR spectroscopy, however, is time consuming since the technique is not typically amenable to automation and the instrument requires samples to be relatively adulterant free, often requiring some sample preparation.

We report on an instrument that links gas chromatography to infrared spectroscopy to allow an automated approach to the IR analysis of drug samples. The technique abandons the “classical” light pipe approach for a direct deposit technique which focuses the GC effluent on a ZnSe window cooled with liquid nitrogen. The window moves to allow discrete sampling of the eluted components by FT-IR. Inclusion of an autosampler allows unattended automated analysis. The instrument yields excellent IR spectra and has good overall sensitivity for drugs of interest. The instrumental design and spectra from a variety of compounds will be discussed.

Using a Single Nucleotide Polymorphism Assay to Differentiate Skeletal Remains From Past Military Conflicts

WADHAMS, Mark J., Rebecca S. Just, Michael D. Coble, Thomas J. Parsons, Carla D. Paintner, Jennifer E. O’Callaghan, Jessica C. Spangler, Timothy P. McMahon, Suzanne M. Barritt-Ross, and Louis N. Finelli
USA AFDIL, 1413 Research Blvd., Rockville, MD 20850

The Armed Forces DNA Identification Laboratory (AFDIL) was established in 1991 for the purpose of using novel DNA technology to identify recovered skeletal remains from past military conflicts. Remains encountered in AFDIL casework have a post-mortem interval in the range of ~30 to 65 years and thus are typically too degraded for standard STR typing. In these cases mitochondrial DNA (mtDNA) sequencing of the remains and comparison to mtDNA profiles from maternal relatives is routinely used for identification. In some instances, however, we encounter matching or nearly matching hypervariable

(HV) region profiles from multiple reference families, thus preventing identification. This is due to the presence of several common HV haplotypes, one of which is observed in over 7% of the Caucasian population. The development of a single nucleotide polymorphism (SNP) assay that utilizes sites within the control region outside of the two hypervariable regions (HVI/II) and in the coding region of the mtDNA genome, increases the discriminatory power of the mtDNA. An 11-plex SNP assay (Panel "A") has been applied successfully in AFDIL casework to discriminate skeletal elements that could not be differentiated on the basis of HVI/II sequence data. This SNP assay will allow AFDIL to make more identifications in cases that would otherwise remain unresolved. The utility and limits of this technology will be discussed, along with case specific examples and the validation of other SNP assays at AFDIL.

Beyond Samples: Track and Control Everything that Affects Quality
HARTEN, Bill, and Carl Hull
UNIConnect LC, 888 West 2000 So., Woods Cross, UT 84087

A great evidence tracking system must do more than log samples and outcomes in a database. Sample quality and integrity depends on the quality of every element affecting the process, or every link in the chain of custody. Catching and preventing problems requires tracking intermediate containers, thorough validation, and capturing required information at each step along the way. People, reagents, and instruments require their own processes, traceability, and controls to ensure the highest quality and confidence. Authorization and training, instrument calibration and maintenance, and reagent QC and inventory are a few of the sub-processes that benefit from effective tracking and integrated control.

This presentation explains the quality factors we can track, how control is applied and how every sample has its own chain of custody history that is created and stored forever. Modifying the tracking system to meet the unique requirements of every forensic lab will also be addressed.

Optimizing Blood Alcohol
RUPPEL, Tim
Perkin Elmer, 710 Bridgeport Ave., Shelton, CT 06484

Blood Alcohol Content (BAC) is often analyzed by headspace/gas chromatography. The variables in method parameters will be discussed toward the goal of method optimization. Parameters of headspace will specifically focus around balanced pressure sample introduction headspace (PerkinElmer). Many parameters will be similar if using GSV headspace instruments (Agilent and Tekmar). Parameters on the GC end of the analysis will be discussed also. Requests of the author from many forensic laboratories have resulted in upgrading blood alcohol applications. Examples will be presented showing higher throughput and routine screening of other analytes such as sniffer inhalants and ethylene glycol. As time permits, a short discussion will follow on headspace/GC without the use of pressurized cylinders.

More on Matching Matches
HOPEN, Thomas J., Chris Taylor, Larry Peterson, and Walther Rantanen
ATF Forensic Sciences Laboratory, US Army Criminal Investigation Laboratory, Georgia Bureau of Investigation, Integrated Paper Services, Inc.

Matching Matches, Part 1, was presented last year at the Joint Orlando and this presentation dealt mainly with the examination and comparison of paperbook matches based on their physical characteristics. This presentation will address a supplemental physical feature useful when conducting a match examination which was not addressed in Part 1. In addition, this presentation will discuss examination and comparison of matches by PLM, SEM-EDS, XRF, TLC, microspectrophotometry, as well as the use of Photoshop® in the comparison of the match stem color.