



Midwest Forensics Resource Center

Research and Development

Program Summary

June 2005

Acknowledgement

These research projects were funded by the National Institute of Justice, through the Midwest Forensics Resource Center at Ames Laboratory under interagency agreement number 2002-LP-R-083. The Ames Laboratory is operated for the US Department of Energy by Iowa State University, under contract No. W-7405-Eng-82.

Midwest Forensics Resource Center (MFRC)

Research and Development Program

Introduction

The mission of the MFRC Research and Development Program, is to provide technological advances in forensic science for the benefit of our regional partners as well as the forensic community at large. Key areas of forensic science need are identified through our interactions with our Midwest partners and our R&D advisory group, as well as through our participation in national meetings in forensic science. Under the sponsorship of the National Institute of Justice, the MFRC solicits proposals for the development of practical and useful technology, instrumentation, and methodology that address needs in areas related to forensic science and its application to operational crime laboratories. The MFRC facilitates proposal development by working to establish partnerships between researchers and our regional partners. The MFRC administers a peer-review of the proposals and then funds the selected projects at a cost of approximately \$55,000 each, with a 12-month period of performance.

The process for selection of these projects includes the following steps: 1) drafting of a call for proposals by MFRC staff, 2) review of the draft call by members of the R&D advisory committee, 3) review and approval of the call by NIJ, 4) issuance of the call to ISU, Ames Laboratory, regional partners, and research organizations, 5) receipt of proposals, 6) review of proposals by R&D advisory committee, 7) ranking and selection by MFRC staff using advisory committee reviews, with concurrence by NIJ, 8) notification of proposers, 9) receipt and review of progress reports by MFRC, 10) receipt and review of final reports by MFRC, R&D advisory committee, and NIJ.

The decision to fund any specific project is based upon a peer-reviewed call-for-proposal system administered by the MFRC. The reviewers are crime laboratory specialists and scientists who are asked to rate the proposals on four criteria areas including: 1) relevance to the mission of the MFRC, 2) technical approach and procedures, 3) capabilities, teaming, and leveraging, and 4) implementation plan. A successful proposal demonstrates knowledge of the background for the research and related work in the field and includes a research plan with a defined plan to implement the technology to benefit our partners at the crime laboratories.

Program Summary Technical Sheets

The following project summaries are meant to demonstrate the range of research funded by the MFRC including chemistry, DNA, and patterned evidence. The project summaries describe the forensic need the projects serve as well as the benefits derived from the technology. The summaries provide a brief description of the technology and the accomplishments to date. In addition, the collaboration with regional partners and the status of the implementation of the technology are highlighted. These technical summaries represent the development and implementation of practical and useful technology for crime laboratories that the MFRC hopes to accomplish.

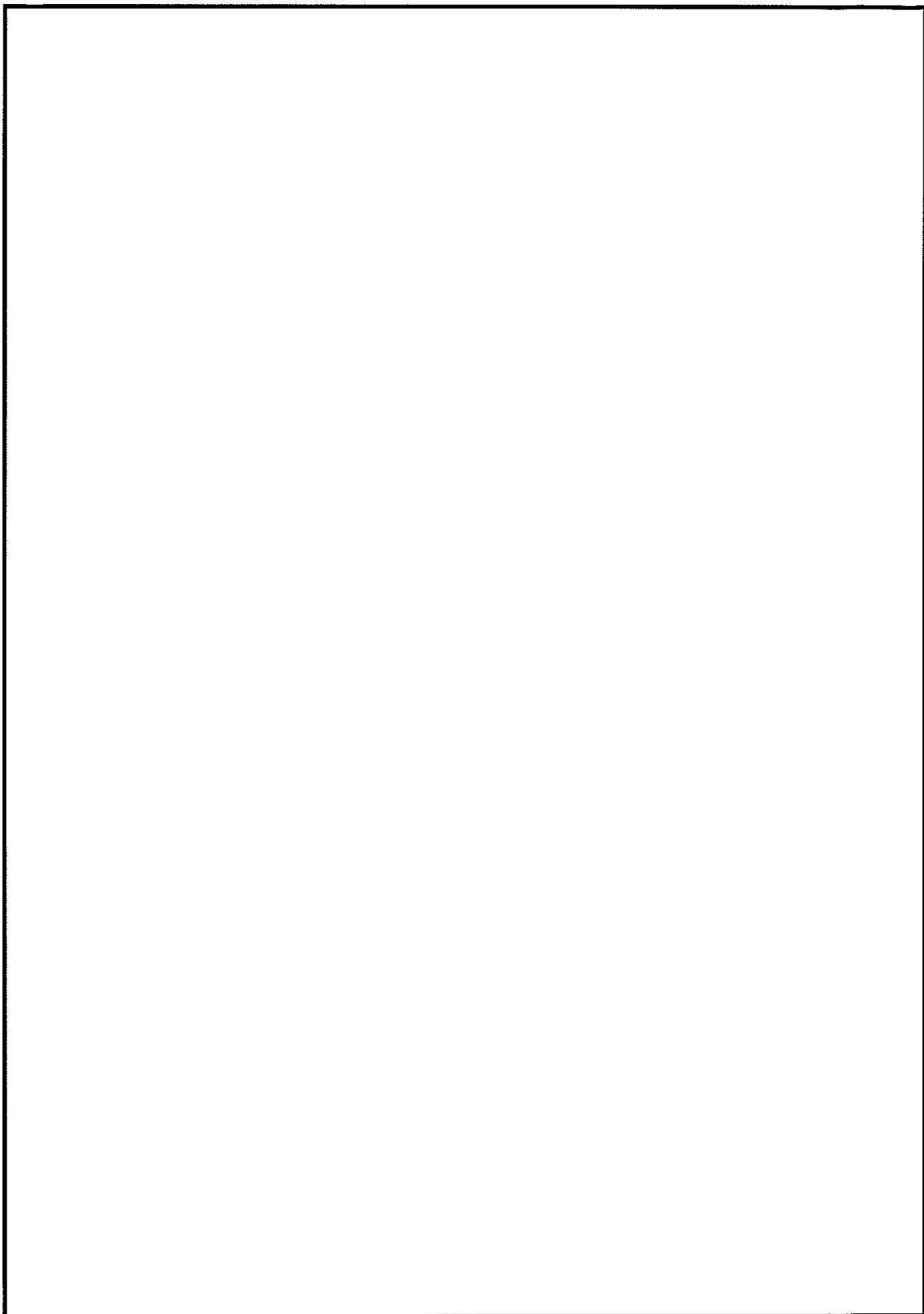


TABLE of CONTENTS

PATTERNEDE EVIDENCE

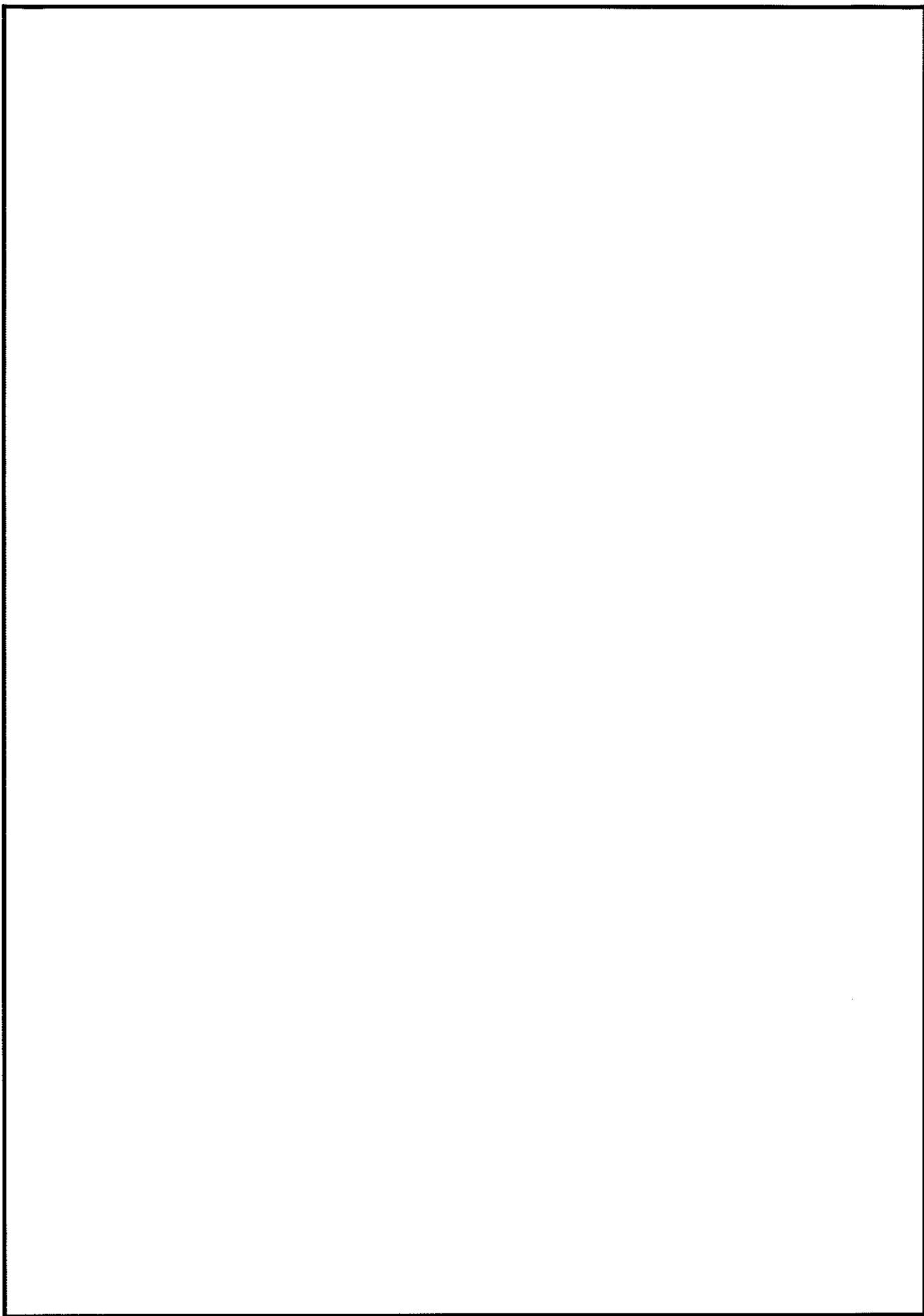
Hall-Effect Measurements Under Alternating-Current Excitation for the Reconstruction of Obliterated Serial Numbers in Magnetic Steels	1
Magnetic Particle Recovery of Serial Numbers	3
Quantitative Characterization of Machining Marks for Comparative Identification	6
Quantitative/Statistical Approach to Bullet-to-Firearm Identification Using Consecutively Manufactured Barrels	8

DNA

Evaluation of the PROMEGA DNA IQ™ System and Qiagen EZ1™ System as an Extraction Method for Mitochondrial DNA Analysis	12
Validation of DNA Quantitation and Species Tests	15
Validation of Y-Plex 12 Database Study	19

CHEMISTRY

Developing Aptamers to Methamphetamine as Nucleic Acid Sensors	22
Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry of Forensic Glass Samples	25
Forensic Analysis of Trace Explosives	28



HALL-EFFECT MEASUREMENTS UNDER ALTERNATING-CURRENT EXCITATION FOR THE RECONSTRUCTION OF OBLITERATED SERIAL NUMBERS IN MAGNETIC STEELS

FORENSIC TECHNOLOGY NEED

Existing forensic techniques employed to recover obliterated serial numbers fall mainly into one of two categories, those requiring extensive sample preparation including the use of acid etchants, and those utilizing magnetic particles to image irregularities in surface magnetic properties. The former is time consuming and utilizes potentially harmful chemicals while the latter messy and potentially low in sensitivity. A new approach is being investigated whereby the stray magnetic field from a magnetized sample is measured using a Hall effect sensor. The sample is magnetized using an electromagnetic c-core yoke. For increased sensitivity, an AC approach is being utilized that benefits from the use of a lock-in amplifier. The approach has been tested on some artificial specimens and part of a real gun with the serial numbers removed by surface grinding.

TECHNOLOGY DESCRIPTION

The objective of this work is to develop a magnetic imaging technique for the nondestructive restoration of obliterated serial numbers in ferromagnetic metals. This can be achieved by imaging the magnetic signatures that result from residual plastic deformations. To obtain both the high sensitivity and high spatial resolution necessary for recovering the serial numbers, advanced Hall-effect sensors based on indium-antimonide technology have been utilized. By scanning the Hall sensor across the obliterated serial numbers it is possible to obtain a two dimensional image of the stray magnetic field distribution, and because of magnetoelastic coupling, this image contains information about the plastically deformed regions under the stamped characters and can therefore be used to reconstruct the serial numbers.

In order to build up an image of the scattered field from a magnetized specimen it is necessary to scan a solid state field sensor (a Hall device, for example) over the surface of the specimen while acquiring readings. This is achieved by mounting the Hall Device on an x-y linear motion stage sitting just above the specimen. The Hall device is mounted on a cantilever to ensure consistent surface contact. Typically, Hall sensors have better performance when operated in constant-current mode. A magnetic imaging system for serial number reconstruction has been assembled and tested in a laboratory environment. Some simple tests have shown the technique to be effective at serial number reconstruction. In many cases the magnetic signature as detected by Hall or GMR sensors is stronger than the visual image provided by techniques such as magnetic particle imaging, Figure 1 for example. The approach was also applied to some more realistic specimens. The results from a shot gun are shown in Figure 2.

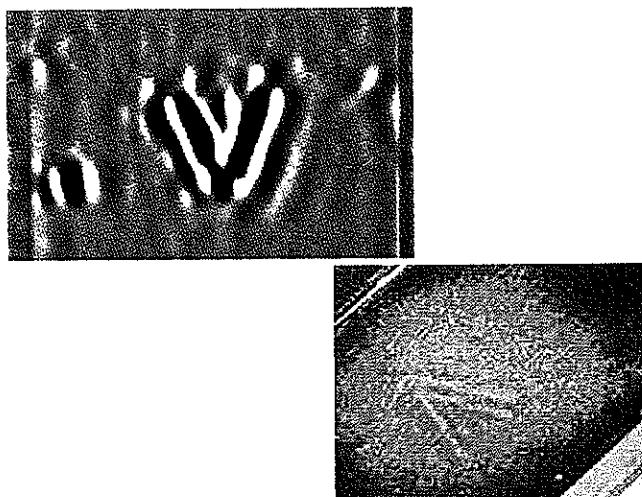


Figure 1. Top Left: Reconstructed image of the letter "V" following a Hall-device scan. Bottom Right: Reconstruction of the same character using fluorescent magnetic aerosol particles following magnetization of the part in a high-current DC coil.

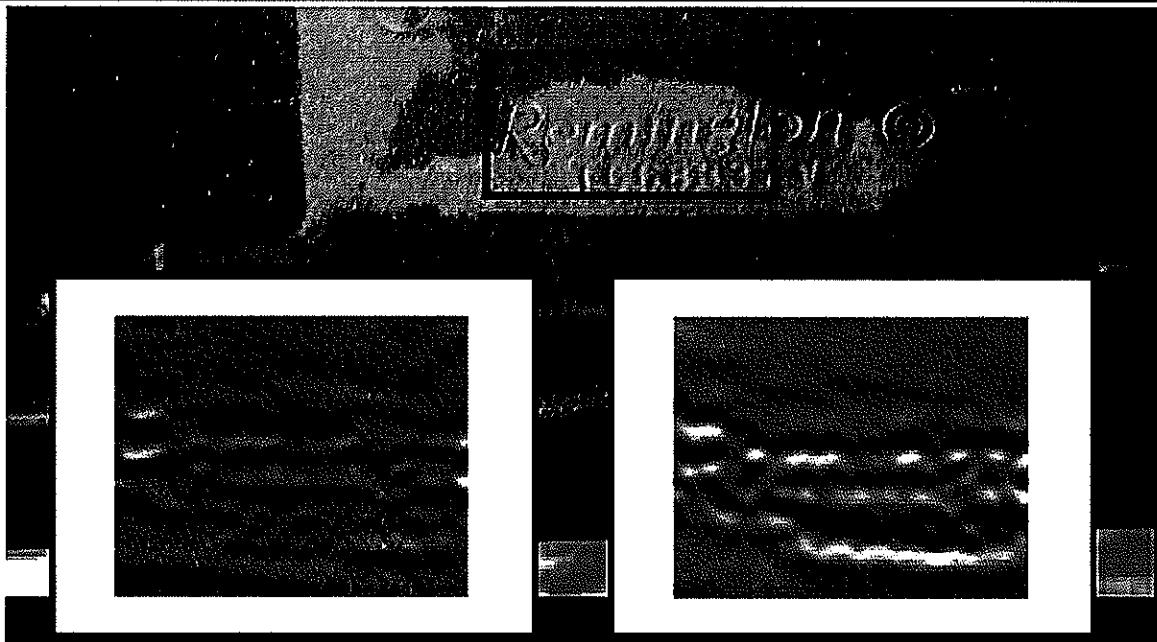


Figure 2. MAIN IMAGE: The manufacturer's name reconstructed using a magnetic particle solution. INSETS: Reconstruction of the same character using magnetic imaging, the in-phase and quadrature components (left and right) are considered separately.

TECHNOLOGY BENEFITS

- Improved sensitivity through the use of advanced Hall sensors. This could mean detection of serial numbers following significant material removal or in metals that are weakly magnetic.
- Improved reliability through automated image-analysis procedures.
- Greater versatility through customizable instrumentation, i.e. ability to tune measurement conditions in order to maximize sensitivity for a particular material or geometry.

COLLABORATION/IMPLEMENTATION

Publications and Presentations

- Johnson, M.J., C.C.H. Lo, and L. Naidu, "Hall-Effect Measurements Under AC Excitation for the Reconstruction of Obliterated Serial Numbers in Magnetic Steels," *Review of Progress in Quantitative Nondestructive Evaluation*, Vol. 23, New York: AIP, 2004: 1445
- This work has been largely conducted in the laboratory and to date no field trials have been carried out.

ACCOMPLISHMENTS/ STATUS

Results to date demonstrate the potential of the Hall-device based magnetic imaging system for serial number reconstruction. Issues remain with the ability to resolve serial numbers that are close together. One way to improve results would be to use a Hall device with a smaller active area such as 50 by 50 mm.

CONTACTS

Dr. Marcus Johnson
Center for Nondestructive Evaluation
285 ASC Building II
1915 Scholl Road
Iowa State University
Ames, IA 50011-3042
(515) 294-6802 fax: -7771
marcusj@iastate.edu

Dr. Chester Lo
Center for Nondestructive Evaluation
205 Metals Development Bldg.
Ames Laboratory
Iowa State University
Ames, IA 50011-3042
(515) 294-4312 fax: -8727
clo@iastate.edu

MAGNETIC PARTICLE RECOVERY of SERIAL NUMBERS

FORENSIC TECHNOLOGY NEED

One method used by crime labs to recover obliterated serial numbers in steel firearms is the magnetic particle technique. The use of this method is based on the detection of metal deformation present under stamped serial numbers after the visible stamp has been removed. The purpose of this project was to define conditions that would increase the likelihood that the technique could be successfully applied in forensic work.

This work was performed at Ames Laboratory's Center for Non Destructive Evaluation in collaboration with the Iowa Division of Criminal Investigation (DCI). Tests were made on actual firearm components provided through the assistance of the DCI.

TECHNOLOGY DESCRIPTION

The aim of this project was to investigate specific aspects of magnetic particle inspection for serial number recovery. This includes tests to understand the magnetic characteristics of different firearm steels that affect their performance in the test, such as varying results for carbon steels and alloy steels after different thermal and forming treatments. Also investigated were the effects of the nature of the sample magnetization (AC, rectified DC, and true DC) and the effects of various detection media, such as visible powders and fluorescent sprays, on test outcome. Surface preparation of the firearms prior to number recovery work was examined.

Visual observations were made after magnetization of the samples and the application of the magnetic suspension. The samples were tested in a horizontal wet magnetic stand using a large coil, as well as a portable yoke. Both AC and rectified DC excitation to the coils were used initially. Early

results on metal test samples indicated that the use of AC current provided inferior results. Serial number and name recovery were then made by visual observation with actual firearms. Both the serial number and part of the "Remington" name were ground away from a Remington 12-gauge shotgun using an electric grinding tool. Recorded images were made using a Minolta DiMAGE 7 digital camera as shown in Figures 1-4.

TECHNOLOGY BENEFITS

This study showed that serial number recovery would be improved by:

- Using fluorescent magnetic particles in the recovery attempt,
- Using DC power supply for control of the magnetic yokes used,
- Modifying the design of such yokes to permit better contact with firearms,
- Performing some surface preparation prior to magnetic particle recovery attempts,
- Attempting magnetic particle recovery first, avoiding damage to the surface through chemicals,
- Using digital photography to capture images.

COLLABORATION/ IMPLEMENTATION

This work was performed at the Iowa Demonstration Lab of the Center for Non Destructive Evaluation at Iowa State University/ Ames Laboratory. Discussions on the merits of this work have been held with Vic Murillo and Bob Harvey of the Division of Criminal Investigation crime laboratory in Des Moines, Iowa and with John Collins of the DuPage County crime laboratory in Wheaton, Illinois. The final project report is posted on the MFRC website for dissemination to crime laboratory partners.

Publications and Presentations

- Utrata, D. and M.J. Johnson., "Magnetic Particle Recovery of Serial Numbers," *Midwest Forensics Resource Center/Ames Laboratory Final Report*. Ames, IA: Ames Laboratory, 2004, IS-5159.
- Utrata, D. and M.J. Johnson, "Development of Magnetic Particle Method for Forensic Recovery of Serial Numbers," *Review of Progress in Quantitative Nondestructive Evaluation*, Vol. 23, edited by D.O. Thompson and D. E. Chimenti, Melville, New York: American Institute of Physics, 2004: 1438-1444.

ACCOMPLISHMENTS AND ONGOING WORK

Several conclusions were reached during the evaluation of the magnetic recovery of obliterated serial numbers. These included: DC power should be used for magnetization, giving better results; the use of fluorescent particles and ultraviolet lighting provided enhanced viewing of recovery efforts; and that some degree of surface preparation improved efficacy of recovery results.

CONTACTS

Mr. Dave Utrata
Center for Nondestructive Evaluation
177 ASC Building II
1915 Scholl Road
Iowa State University
Ames, IA 50011-3042
(515) 294-6095 fax: -7771
heydave@iastate.edu

Dr. Marcus Johnson
Center for Nondestructive Evaluation
285 ASC Building II
1915 Scholl Road
Iowa State University
Ames, IA 50011-3042
(515) 294-6802 fax: -7771
marcusj@iastate.edu

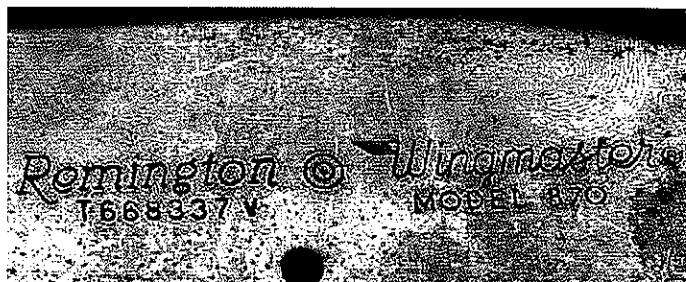


Figure 1. Original "Remington" name and serial number before obliteration.

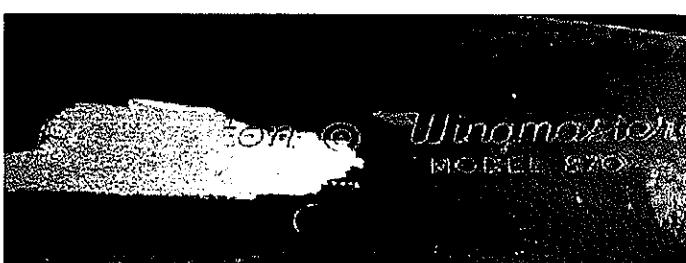


Figure 2. The "Remington" name and serial number after obliteration.

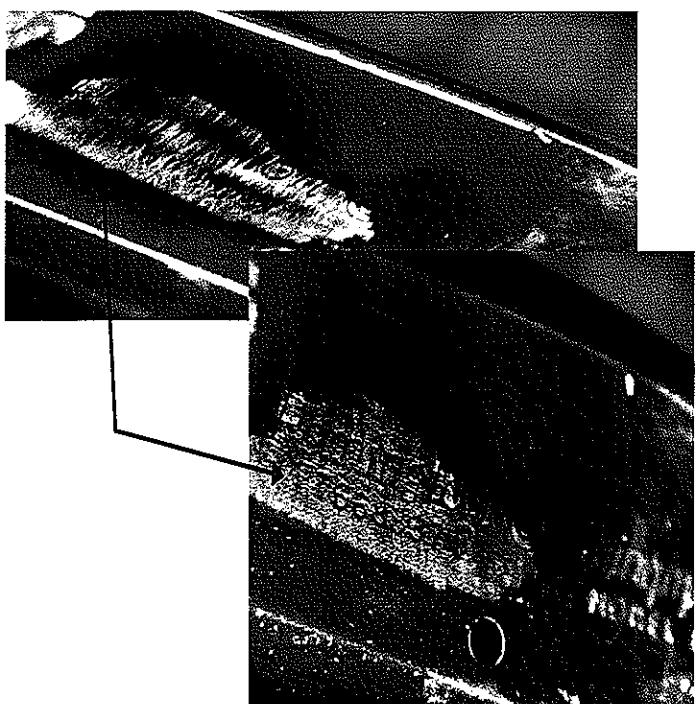


Figure 3. Magnetic images after testing in a yoke using "AC" power.

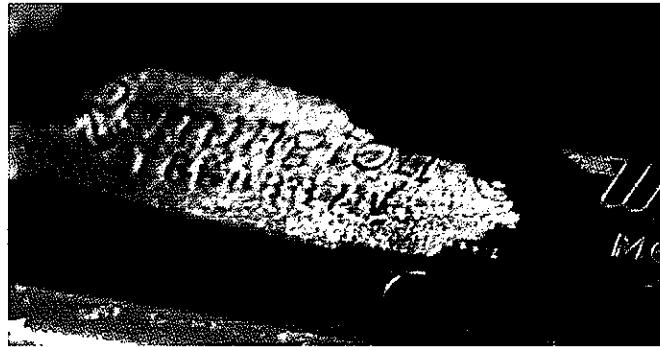
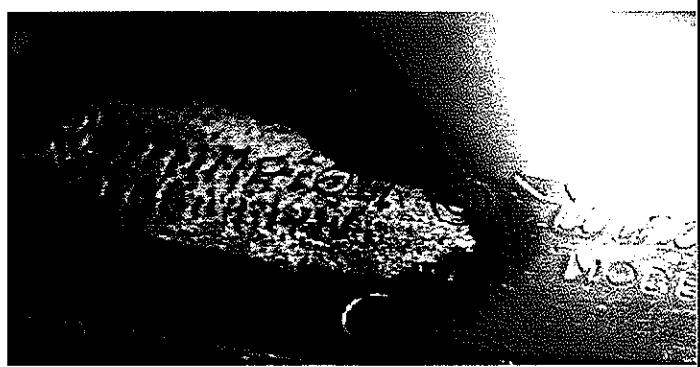


Figure 4. Magnetic images after testing in a yoke using "DC" power.

QUANTITATIVE CHARACTERIZATION OF MACHINING MARKS FOR COMPARATIVE IDENTIFICATION

FORENSIC TECHNOLOGY NEED

The analysis of tool marks at a crime scene present a special challenge in that the mark left is a function of not only what kind of instrument but is also a function of how that instrument was used by a particular individual. Toolmarks are difficult to characterize in that, unlike bullets or cartridge cases where the geometry is fairly predictable, they present a wide range of possible shapes and sizes. The force applied and the angle of attack create differences in the appearance of the markings. This project seeks to extend the quantification of tool marks from two-dimensional to three-dimensional examinations.

This work was performed by the Ames Laboratory in collaboration with Jim Kreiser, retired forensic scientist of the Illinois State Police Crime Laboratory.

TECHNOLOGY DESCRIPTION

The objective of this project is to develop a method to quantitatively measure surface roughness as a means of identifying features such as toolmarks. The method will be applicable to any shaped surface and will involve a statistical analysis of the data to determine the probability of a match between an unknown and a standard sample. The overall goal is to provide local, state, and federal law-enforcement officials with statistically valid data that is suitable for courtroom presentation.

A series of screwdrivers were purchased and both sides were used to produce markings on a series of small brass and lead plates (all samples were produced by Jim Kreiser). Often the toolmark may be left on the surface of a door or some structural member, making it impossible to remove the actual evidential mark for examination. In such cases replicas

are made of regions of interest, typically using either an epoxy or silicone based resin. These replicas can then be removed and optically examined and compared to similar markings produced using suspected tools. Commercially available material was used to produce replicas of the surface. The surface relief of both the replicas and the original marks was examined using a Detroit Precision Hommel profilometer. The surface profile was found by moving a delicately balanced diamond stylus across the surface of the toolmark perpendicular to the mark. Height measurements are taken at periodic intervals and the data output of the profilometer is obtained as an array (or matrix). Normally, a three-dimensional image will be produced by making a series of parallel passes across the surface, a typical scan involving up to 6000 lines with 9600 data points along each line. The collected data were analyzed using a computer spreadsheet.

Once a working routine was in place it was tested by comparing scans between known match and non-match areas as identified by Mr. Kreiser (Figure 1). In over 90% of the cases the known match areas were identified with a high degree of confidence.

TECHNOLOGY BENEFITS

- The process yields a forensic technique that is rapid, easy to perform, applicable to any shaped surface, and has the potential for automated matching
- This method provides quantitative surface data to answer challenges posed by Daubert. The current development of a computer match routine will take the quantitative data, automatically align the 'evidence' and 'standard' scans, and then compare them statistically.

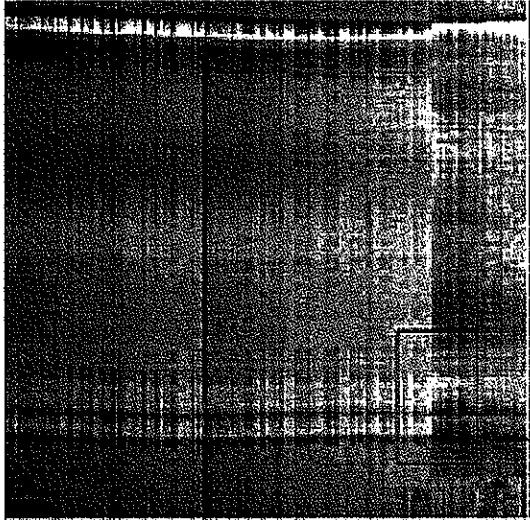


Figure 1. Plot of depth data obtained from scans (each scan presented vertically here) of the profilometer across the evidence sample (left) and the standard sample (right). This data is presented with the scan data from the two samples abutting along the discontinuity at the right portion of the image, to simulate the hairline in a stereomicroscope. The area indicated in the box corresponds to the boxed area in Figure 2.

COLLABORATION/ IMPLEMENTATION

All samples were produced by Jim Kreiser, retired forensic scientist of the Illinois State Crime Laboratory.

Publications and Presentations

- Chumbley, L.S., L.J. Genalo, "Quantitative Characterization of Machining Marks for Comparative Identification," *Midwest Forensics Resource Center/Ames Laboratory Final Report*, Ames, IA: Ames Laboratory, 2004, IS-5165.
- Bossard, C.A., L.S. Chumbley, L. J. Genalo, and M.F.Besser, "Examination of Toolmark Replicas Using a Surface Profilometer." *Journal of Forensic Science*, 50 (January 2005):143-156.
- Chumbley, L.S. and L.J. Genalo, "Quantitative Characterization of Tool Marks for Comparative Identification", *Proceedings: American Academy of Forensic Sciences*, 10, Paper D31, Denver, CO: Publication Printers Corporation, 2004: 170.

ACCOMPLISHMENTS AND ONGOING WORK

The initial results were used as the basis for a proposal submitted and funded by the National Institute of Justice to continue work on the routine for comparing scans between known matched and unmatched surface areas. A paper summarizing the initial results was submitted to the *Journal of Forensic Sciences*.

CONTACTS

Dr. L. Scott Chumbley

Professor

Materials Science & Eng. Dept.
Iowa State University/Ames Lab
214 Wilhelm Hall
Ames, IA 50011
(515) 294-7903 fax: -4291
chumbley@iastate.edu

Dr. Larry Genalo

Professor

Materials Science & Eng. Dept
Iowa State University/Ames Lab
2220A Hoover Hall
Ames, IA 50011
(515) 294-5444 fax: -4722
genalo@iastate.edu

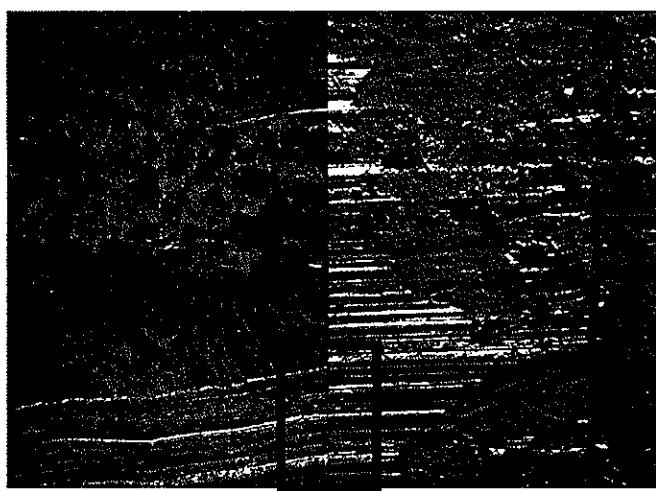


Figure 2. Small toolmark match region between an evidence sample on the left and a standard sample on the right identified by an examiner using comparison microscope.

Quantitative/Statistical Approach to Bullet-to-Firearm Identification Using Consecutively Manufactured Barrels

FORENSIC TECHNOLOGY NEED

Firearms identification is one of the "identification" sciences that rely on pattern comparisons to reach conclusions as to identity or difference. There is very widespread agreement on the validity of the identification sciences and on their conclusions. However, some challenges to these "identification" or pattern-evidence sciences have been mounted since the U.S. Supreme Court handed down its decision in *Daubert v. Merrell Dow Pharmaceuticals* [1]. The challenges do not so much allege that pattern evidence cannot be individualized as that there is not an appropriate, objective scientific basis for the assertion that it can. This situation has prompted research that will help establish on a firm, scientific basis for what most forensic scientists already think is true – that pattern evidence can be shown to be individualizable.

In the firearms identification arena, these more recent studies [2-6] take different approaches, but all are focused on establishing generalizable principles. There have been some prior studies on consecutively manufactured rifled barrels. However, none of

the prior studies have taken advantage of the analytical tools afforded by either the SciClops scanning system or densitometric conversions of IBIS images. The data gathered by these systems can be analyzed using appropriate non-proprietary software, such as Un-Scan-It, SigmaScanPro, PeakFit, etc. Analysis of data is similar to analyzing similarities / differences in gas chromatograms, mass spectra, or other familiar patterns. Data analysis permits the application of appropriate statistics to help establish the expected low probabilities of "chance match" in comparison with the size of the test population.

TECHNOLOGY DESCRIPTION

The specific aim of this project was the application of quantitative scoring methods to the striation markings used by firearms examiners for bullet-to-firearm identifications to obtain data in a format amenable to frequency/statistical analysis. This approach used consecutively manufactured barrels from two different manufacturers, that is, under conditions where maximal similarity in the identification markings is expected. Bullets fired from consecutively manufactured barrels on the same assembly line should be as similar as two bullets that were fired from different barrels can be. The idea is that if these bullets can be told apart, there should not be a problem differentiating bullets from barrels that are expected to be different.

This project tested the prospect of producing a quantitative picture of similarities / differences among the striation markings normally used by firearms examiners in determining whether a particular bullet was fired by a particular firearm (with a rifled barrel). This method could help identify an optimal approach to generating objective data that attempts to partially simulate the pattern identification process, and thus help provide a scientifically defensible basis for *Daubert*-type challenges.

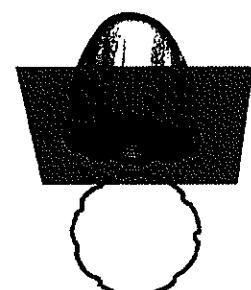


Figure 1. Schematic of the scanned circumference of the bullet.

The goal of this project was to determine whether instrumental analyses of the individual striation markings on fired bullets, or images of fired bullets, could yield quantitative data that mirrored the comparisons examiners perform with their eyes. Figure 1 shows a schematic of a scanned bullet with striation markings around its circumference. This project was designed to help validate firearms identification by instrument analyses under *Daubert* guidelines. Two approaches to scanning bullet striation markings were used and the results compared. First, fired bullets were imaged using the (Forensic Technology, Inc.) FTI IBIS system, the standard imaging device for the NIBIN database. Second, fired bullets were analyzed using a measuring device called SciClops^â, manufactured by Intelligent Automation Inc. (Figure 2). A third task consists of a validation test by human firearms examiners.

The experiment which fired bullets from consecutively manufactured barrels has been done before with human examiners, and yielded 100% correct identifications. The prior study was done with 9 mm bullets. We chose .45 cal for our study. The expectation was that human examiners could correctly match up even these extremely similar, yet different, bullets in a blind trial type test. We explored whether a measuring device that attempts to measure what examiners look at, or an image scan analysis of bullet surface scans, could demonstrate quantitatively what examiners do by eye – getting “match scores” of some kind that were higher among bullets fired from the same barrel than from those fired from different, or even consecutively manufactured, barrels.

TECHNOLOGY BENEFITS

This project looked at validation approaches that might satisfy the criteria of the *Daubert* decision. These can be summarized as: Is there a scientific basis for the proposition? The proposition, in this case, is that bullet to barrel matching is possible and accurate. To test the proposition, one turns to examples of the pattern that are maximally similar among the “different” category. With fingerprints, for



Figure 2. The SciClops laser-based profilometer by Intelligent Automation, Inc.

example, one might collect the prints of identical twins. With fired bullet evidence, the correlate is bullets fired from consecutively manufactured barrels.

The primary purpose of IBIS is to collect and store images of the land impressions on bullets, and the firing pin indentation and other markings on cartridge cases. Given a barrel model with some number of lands, say six for example, there would be six images associated with an exemplar bullet from that barrel – one image for each land impression (sometimes called a land engraved area or LEA). A questioned bullet can then be imaged, and the image compared with existing image files. If the questioned bullet is in good condition, and six land impressions can be imaged, and they can each be pair-wise compared with the six on any exemplar. The system thus enables connecting fired evidence from different cases, and in this way assisting investigations. It is not and has never been intended to substitute for a human firearms examiner, but only to sort through large quantities of evidence and generate a manageable list of “possibles.”

When two bullets are compared in the IBIS system, three different scores result. These are max phase, peak phase, and peak score. On bullets with, for example, six twist and therefore six LEAs, there are 36 comparison scores: LEA1 of bullet 1 with LEA1 of bullet 2, then LEA2 of bullet 2, then LEA3 of bullet 2, and so on. The highest score here will indicate that the

proper LEAs are being compared (highest peak phase). Then a peak score is computed, and the highest peak scores among a group of bullets being compared are noted.

Once all bullets are entered, a correlation is performed for each bullet against all other bullets in the test group. For each bullet a correlation result is produced. For each correlation result produced, the three score arrays noted above are generated. Each score array is sorted, and knowing which bullet is the "sister," each array is consulted to determine its position. Out of the three arrays the lowest position is noted. And the lowest position occupied among all the arrays is recorded. From these data, a probability density vs. position can be computed – the probability that the sister bullet of any given bullet being compared will be one of those in the top n positions, where n is the position number being plotted – indicating a perfect match.

This study showed that the FTI IBIS image comparison can correctly distinguish between all Springfield barrel bullets. The comparison results provide some evidence that barrel to bullet matching is objectively reliable.

COLLABORATION/ IMPLEMENTATION

There are other, independent efforts underway to help establish a sound scientific basis for the comparison of primary pattern evidence types: bullet and cartridge case markings, fingerprints, and handwriting. We collaborated with (Forensic Technology, Inc.) FTI, the manufacturer of IBIS systems for the imaging and image-databasing of fired evidence (bullets and cartridge cases). FTI agreed to image the study bullets, and provided bitmap image results. To protect their technology, they could not share the details of their comparison algorithms.

For the human examiner validation test, we recruited examiners to do 55 tests. The expectation was that human examiners would be able to make identifications with most of the Q specimens (i.e., match the Q to a K) and that there would be no mis-identifications. Indeed, the human examiner validation test

results showed that there were no mis-identifications. This result on .45 cal. bullets confirmed the earlier "consecutively manufactured barrel" exercise with firearm examiners on 9 mm bullets.

Publications and Presentations

- Striupaitis, P. and Gaenslen, R.E. "Quantitative /Statistical Approach to Bullet to Firearm Identification with Consecutively Manufactured Barrels-Final Report," *Midwest Forensics Resource Center/Ames Laboratory Final Report*, Ames, IA: Ames Laboratory, 2004, IS-5170.

ACCOMPLISHMENTS AND ONGOING WORK

Probability density versus position for Springfield bullets was generated using the IBIS Technology image comparisons. This calculation gives the probability that the sister bullet of any given bullet being compared will be one of those in the top n positions, where n is the position number plotted – indicating a perfect match. These FTI IBIS image comparison tests correctly distinguished between all Springfield barrel bullets and between most, but not all, of the HiPoint Barrel bullets. In the HiPoint cases that were not distinguished all of the time, they would be distinguished at least 83% of the time. The results provide some evidence that barrel to bullet matching by image comparison is reliable.

The early version of the SciClops measuring system used in this study was not able to fully resolve either manufacturer barrel bullets as well as the FTI IBIS image comparison technology.

References

1. Daubert v. Merrell Dow Pharmaceuticals, U.S. Supreme Court, 509 U.S. 579 (1993)
2. Bachrach B. "Development of a 3-D Automated Firearms Evidence Comparison System." *J. Forensic Sci.* 47 [6] (2002):1-12. [paper # JFS2002054_476]
3. Nichols, RG. "Firearm and Toolmark Identification Criteria: A Review of the Literature." *J. Forensic Sci.* 42 [3] (1997): 466-74.
4. Nichols, RG. "Firearm and Toolmark Identification Criteria: A Review of the Literature, Part II." *J Forensic Sci.* 48 [2] (2003):318-27. [paper # JFS 2002246_482]
5. Brundage DJ. "The Identification of Consecutively Rifled Gun Barrels." *AFTE J* (summer 1998): 30.
6. Miller J. "An Examination of Two Consecutively Rifled Barrels and a Review of the Literature." *AFTE J* (summer 2000): 32.

CONTACTS

R.E. Gaenslen
Forensic Science Group
Dept of Biopharmaceutical Sciences
University of Illinois at Chicago
833 S. Wood St.
Chicago IL 60612
(312) 996-2250
reg@uic.edu

Peter Striupaitis
Firearms Examiner
(Retired from Illinois State Police Forensic
Science Command)
c/o University of Illinois at Chicago
833 S. Wood St.
Chicago IL 60612
(312) 996-0888
striup@aol.com

EVALUATION OF THE PROMEGA DNA IQ™ SYSTEM AND QIAGEN BIOROBOT EZ1™ SYSTEM AS AN EXTRACTION METHOD FOR MITOCHONDRIAL DNA ANALYSIS

FORENSIC TECHNOLOGY NEED

The expanding demand for DNA analyses is creating the need for automation to improve the efficiency and quality of the product, and to save time. The use of automation for amplification and genetic analysis is not new for forensic DNA analysis. DNA extraction and quantitation are two methods for which automation is relatively new. The need to automate the extraction process is two-fold. One need is for the reduction of the time for DNA extraction from a day or two to an hour or less. The DNA analyst would in turn be free to work more cases and reduce case backlogs and have shorter case turn-around times. The second need is to minimize analyst "hands-on" time with the evidence, which will minimize evidence contamination and will reduce the analytical space required for manual extraction methods.

DNA automation is a common desire for many crime laboratories and forensic analysts. The automated systems available can be large-scale and all-encompassing such as the Beckman-Coulter Biomek® 2000 to a small scale extraction system like the Qiagen BioRobot EZ1™. The Northern Illinois Police Crime Laboratory and the DuPage County Sheriff's Office Crime Laboratory have collaborated on the evaluation and application of the Qiagen BioRobot EZ1™, not only as a method for automated DNA extraction of nuclear DNA, but also as an automated method for extracting mitochondrial DNA.

TECHNOLOGY DESCRIPTION

The Qiagen BioRobot EZ1™ System (Figure 1) is a self-contained automated DNA extraction system utilizing magnetic particles contained within individual reagent cartridges. The reagent cartridges are supplied in a



Figure 1. The Qiagen BioRobot EZ1™ System.

sealed condition and are only opened within the instrument, therefore minimizing contamination from the outside environment. Also contained within the instrument are a heating block, a magnet, and a pipette head attachment for dispensing reagents.

Although the Qiagen BioRobot EZ1™ System is versatile to employ various extraction methods for a variety of biological substances, its primary use will be for the extraction of nuclear and mitochondrial DNA from forensic samples.

Presently, the Northern Illinois Police Crime Laboratory, the DuPage County Sheriff's Office Crime Laboratory, and many other forensic laboratories utilize a phenol/chloroform (organic) extraction for the extraction of DNA. This method, although suitable, requires the analyst to manipulate many tubes for the extraction and purification of the DNA, which may lead to potential sample loss or contamination. The organic extraction also utilizes a fume hood to dispel any vapors from the phenol, which requires more space allocation for extraction areas.

The Qiagen BioRobot EZ1™ System has all of the necessary components for the extraction

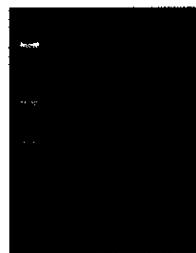
and purification of DNA contained within the system. Minimal "hands-on" time is needed for sample preparation. The procedure requires a 15-minute incubation period before the sample is placed in the robot. Sample incubation for an organic extraction may typically take 6 hours to overnight. All of the necessary consumables (buffer, tips, tubes, and reagent cartridges) are included in the same container. An organic extraction may require various consumables from various vendors. Finally, overall time from placing the sample cutting in a tube to final DNA extraction typically takes 35 to 40 minutes for the BioRobot EZ1™. An organic extraction may take from 1 to 1 ½ days.

TECHNOLOGY BENEFITS

- Contamination and sample loss incidents should be minimized due to DNA extraction and purification in an enclosed system and individual reagent cartridges. Once the sample is placed in the BioRobot EZ1™, DNA extraction and purification is performed in parallel (one sample per one tip per one reagent cartridge).
- Automation of DNA extraction will be most time effective by reducing "hands-on" time an analyst has to spend with a sample. The process of extracting up to six samples at a time can take up to 35-40 minutes using the BioRobot EZ1™ and can be repeated for however many samples need to be extracted. The current organic method typically can take from 6 hours to overnight. Additionally, purification of the DNA by the organic method takes an additional 1-1 ½ hours using separate tubes.

COLLABORATION/ IMPLEMENTATION

The data obtained in the current project was presented as a poster presentation at the 15th International Symposium on Human Identification 2004 sponsored by Promega in Phoenix, Arizona.



Lane 1 Ladder XIV
Lane 2 Blood
Lane 3 Urine
Lane 4 Saliva
Lane 5 Semen
Lane 6 Perspiration

Figure 2. The amplification of the mitochondrial HVI as well as HVI regions of the blood, urine, saliva, semen, and perspiration samples with agarose gel quantification.

Publications and Presentations

- Pfoiser, K., and M.K.Gannon, "Validation of the Qiagen BioRobot EZ1: A Quick, Automated Method for Extracting DNA," Poster Presentation, 15th International Symposium on Human Identification.
- Data from the current project will also be compiled by Qiagen for an upcoming paper. Initial plans are to use the BioRobot EZ1™ for the extraction of nuclear DNA at the Northern Illinois Police Crime Laboratory. Future collaboration with the DuPage County Sheriff's Office Crime Laboratory and the Illinois State Police is being considered for the analysis of mitochondrial DNA.
- Other organizations utilizing the BioRobot EZ1™ with publications for forensic use are from the San Diego Police Department, the Institut für Rechtsmedizin der Charité Berlin, and the Armed Forces Scientific Institute for Protection Technologies.

ACCOMPLISHMENTS AND ONGOING WORK

Primarily, the major accomplishments achieved from this research have been to obtain valuable data for both nuclear DNA and mitochondrial DNA in forensic samples. The body fluid study utilized samples typically tested in forensic casework. Table 1 illustrates the results of the amplification of the nuclear DNA. Figure 2 illustrates an agarose gel

Sample Type	Volume (µL)	Quantity (ng/µL)	STR loci profiled
Blood	100	5.0	13
Urine	100	Below detection	3
Saliva	100	5.0	13
Semen	100	1.25	13
Skin/ Perspiration	N/A	Below detection	13
Buccal swab	N/A	5.0	13

Table 1. The Quantity and STR loci profile results of the amplification of the nuclear DNA for given test volumes of various sample materials.

CONTACTS

Kenneth Pfoser & M. Kelly Gannon
 Northern Illinois Police Crime Laboratory
 1000 Butterfield
 Suite 1009
 Vernon Hills, IL 60061
 (847) 362-0676 fax: -0712
 dna@nipcl.org

quantifying the amplification of the mitochondrial HVI and HVII regions of the blood, urine, saliva, semen, and perspiration samples.

Future work will include the additional validation and utilization of protocols for low copy DNA samples typically tested for mitochondrial DNA (bone, hair, teeth, etc.). Also, a new method for mitochondrial DNA testing by Roche, LINEAR ARRAY Mitochondrial DNA HVI/HVII Sequence Typing Kit will be investigated using samples from the current study.

VALIDATION OF DNA QUANTITATION AND SPECIES TESTS

FORENSIC TECHNOLOGY NEED

DNA Quantification

One of the least precise technical steps in DNA preparation and analysis is quantitation. Originally, both UV absorbance spectroscopy (260/280 nm ratios) and mini-gels were used to estimate the total DNA from a preparation. UV is a tried-and-true method, but is limited by the lower range of the assay (~5 ng/µL) and by the volume required for most cuvettes (~200 µL). Sometimes, there is not enough total sample for this assay. Mini-gel estimates are limited because they rely on visual estimation of fluorescence intensity and are subject to 3- to 5-fold variation. Further, both assays estimate total DNA. In "clean" specimens (DNA prepared from blood cells, for instance), total DNA equals human DNA. But in most forensic specimens, including DNA from buccal or vaginal swabs, there is considerable non-human DNA. What is needed is an estimate of human DNA.

DNA-Based Species Testing

Labs can receive non-human blood or body fluid evidence. Traditionally, dried blood evidence was tested for species of origin by immunological techniques. Some labs still do this. But there is a tendency to do all testing using DNA- or RNA-based methods. It is still important to test evidence for species prior to subjecting it to DNA analysis.

TECHNOLOGY DESCRIPTION

The universally used PCR-amplification kits are validated for 1-2 ng template human DNA. It is probably fair to say that a target range of 500 pg to 4 ng is acceptable – about the same results are expected within that range of input template. Outside this range, PCR artifacts that make interpretation of the profile difficult or impossible start to become a significant factor.

The current widely used method for estimating human DNA is based on a dot-blot technique

contained in the QuantiBlot kit from ABI (Applied Biosystems Group). The estimate relies on a visual comparison of color or luminescence intensity between standards and specimens. We have investigated the validity of BodeQuant, a sensitive, spectrofluorimetric assay for human DNA estimation.

At the outset of our quantitation project, BodeQuant was in use only at Bode (The Bode Technology Group, Inc.), where it was devised. More widespread use requires validation. Sufficient validation by us and by others, and willingness of labs to acquire the necessary hardware, could result in widespread use of this method. Currently, a different technology, based on RT-PCR, is available for human DNA quantification. It is not clear whether BodeQuant would be materially cheaper than RT-PCR, but probably so. In addition to hardware costs, RT-PCR will be done with expensive test kits. There are no test kits marketed for BodeQuant.

Our project was designed to validate the use of a PCR-based amplification of a mitochondrial sequence called Cox-I for species testing. The project was designed as "proof of concept" rather than operational assay. The idea was to demonstrate that a species test could be based on the amplification of Cox-I using fluorescent-tagged primers – the same fluorescent tags used for CODIS loci. If the PCR amplicons for different species were of different size, they would separate as clean peaks by CE in an ABI analyzer. Most labs use such instruments for CODIS locus profiling. This would be just another injection.

TECHNOLOGY BENEFITS

Unless there is some independent reason to know that a bloodstain is human (such as it was observed dripping from a body at a scene), testing is necessary to verify species since it is possible that blood or body fluids from animals

Specimen	BQ Run 1: 22Mar04						BQ 1 Average	BQ Run 2: 29Mar04				BQ 2 Average
	Quantiblot Analyst 1	Quantiblot Analyst 2	Well 1, Scan 1	Well 2, Scan 1	Well 1, Scan 2	Well 2, Scan 2		Well 1, Scan 1	Well 2, Scan 1	Well 1, Scan 2	Well 2, Scan 2	
9A	10.00	10.00	13.07	11.75	13.10	11.57	12.37	11.32	12.64	11.22	12.62	11.95
10A	10.00	8.00	9.76	8.83	9.73	8.78	9.28	8.57	no data	8.69	no data	8.63
11A	8.00	10.00	9.10	7.94	9.02	7.82	8.47	10.21	9.97	10.15	10.02	10.09
12A	2.50	3.00	3.67	3.67	3.78	3.71	3.71	3.16	3.19	3.17	3.18	3.18
MB-A	none	none	0.34	<0.1	0.33	<0.1	N/A	0.12	<0.1	0.18	<0.1	N/A
1B	0.13	0.13	0.61	<0.1	0.59	<0.1	---	0.11	0.12	0.16	0.19	0.15
2B	5.00	5.00	15.98	13.71	16.05	13.80	14.89	5.61	5.73	5.78	5.85	5.74
3B	1.25	1.25	2.02	1.14	2.00	1.13	1.57	1.76	1.44	1.89	1.47	1.64
4B	16.00	12.00	20.78	23.98	20.83	24.33	22.48	10.59	11.99	10.76	12.09	11.36
5B	5.00	5.00	7.21	7.26	7.13	7.20	7.20	6.67	6.95	6.71	7.11	6.86
6B	5.00	5.00	13.06	12.51	13.14	12.67	12.85	5.44	6.06	5.58	6.28	5.84
7B	12.00	12.00	16.64	13.76	16.81	13.89	15.28	15.03	14.64	14.92	14.73	14.83
8B	12.00	10.00	11.55	9.94	11.56	9.89	10.74	9.30	8.79	9.37	8.90	9.09
9B	7.00	8.00	10.78	9.29	10.86	9.20	10.03	8.57	8.92	8.77	9.06	8.83
10B	16.00	14.00	9.77	11.42	9.80	11.40	10.60	11.37	11.04	11.38	11.19	11.25
11B	11.42	9.14	11.98	10.31	12.01	10.37	11.17	10.10	10.79	10.07	10.85	10.45
MB-B	none	none	0.44	0.24	0.46	<0.1	N/A	<0.1	<0.1	<0.1	<0.1	N/A

Table 1. QuantiBlot vs BodeQuant (BQ) (Numbers are DNA quantities in ng/µL)

kept as pets could find their way onto evidential specimens. And in some cases, species is the issue – illegal game killing, for example. In addition, stains found on textiles or other items could be of non-human origin. Our results suggest that this methodology could be developed without much difficulty. Forensic labs are seeking standardization and comparatively easier validation. This approach uses amplicon detection technology identical to Forensic Lab technology for DNA profiling amplicon detection.

COLLABORATION/IMPLEMENTATION

We have collaborated with lab personnel in the Chicago Center of the Illinois State Police Forensic Sciences Command. We are asking the Command's R&D section to explore Bodequant as a possible alternative to QuantiBlot.

We are seeking others with an interest in the species methodology. The data is given in appendices of the final report.

Publications and Presentations

- Gaenslen, R.E. and Llewellyn, B., "Further Development and Validation of DNA-Based Methods for Species of Origin Determination and Human DNA Quantitation in Forensic Casework Specimens—Final Report," *Midwest Forensics Resource Center/Ames Laboratory Report*, Ames, IA: Ames Laboratory, 2005, IS-5169.

ACCOMPLISHMENTS AND ONGOING WORK

DNA Quantification

The standard curve was optimized by titration of the amount of the TH01 primer set used in the PCR. Once the standard curve was optimized, preliminary validation of the assay was initiated. A small number of samples were run with the functional BodeQuant standard curve assay and compared directly with QuantiBlot. The resulting DNA concentrations of each of the specimens were first estimated by running a preliminary yield (mini) gel and

then employing QuantiBlot with chemiluminescence detection on x-ray film. Specimens used for validation – side by side comparison with QuantiBlot – were extracted by the standard phenol-chloroform-isoamyl alcohol isolation method. To determine the variability in the subjective band intensity judgments required by QuantiBlot, DNA quantities were separately determined by the research assistant, and by a DNA analyst-supervisor at the lab (referred to as "Analyst 1" and "Analyst 2"). The results are shown in Table 1.

Although there are some variations in the subjective band intensity judgments of the two analysts with the specimens, the QuantiBlot results are close to the BodeQuant average in all cases. The results in Table 1 show enough differences that further validation would be necessary before BodeQuant replaced QuantiBlot as the sole quantitation technique. The data suggest that it probably could be accomplished, however.

QuantiBlot may have an advantage in that it has been used extensively for a number of years. Yet, even our limited data show that there is variability in the estimates resulting from the subjective judgment of band intensity in QuantiBlot.

In practice BodeQuant is faster than QuantiBlot in the sense that the analyst has down time during BodeQuant to be doing other things. In addition, more specimens can be run, and the range of the assay is greater. Another advantage to BodeQuant is that the assay must be standardized using curve fitting methods. This characteristic of BodeQuant provides a statistical basis for the results. We followed the lead of the original paper [Fox, J.C., C.A. Cave, J.W. Schumm, *BioTechniques*, 314-22(2003)], and used Microsoft Excel for this purpose.

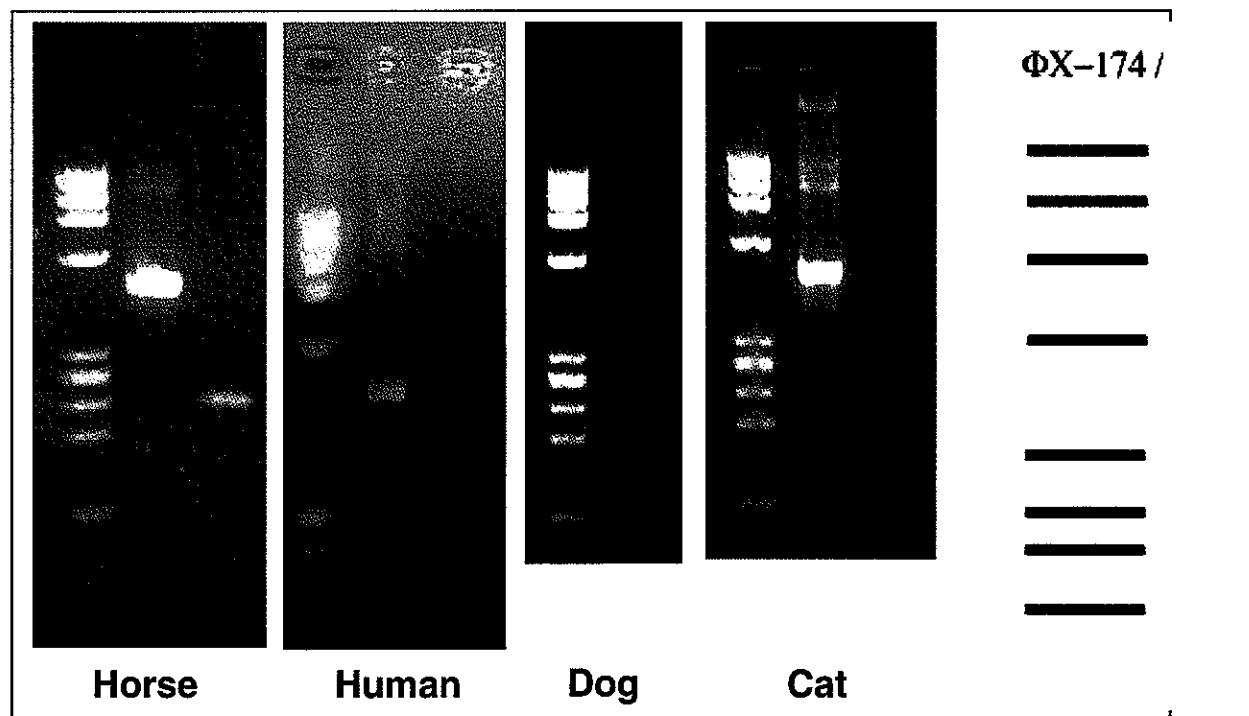


Figure 1. Composite Photos of Post-Amplification Gels for All Four Species. The left-most lane is a Φ X-174-HaeIII ladder. To the right is a diagrammatic representation of the sizing bands in this ladder. The ladder bands do not always resolve completely, but it is generally possible to verify that the size of the amplicon is approximately as expected. In the Horse, Human and Cat gels, the middle lane is a λ -DNA PCR control product of 500 bp. Because it is genomic, the number of PCR cycles used for the mt-DNA result in overamplification of the λ control.

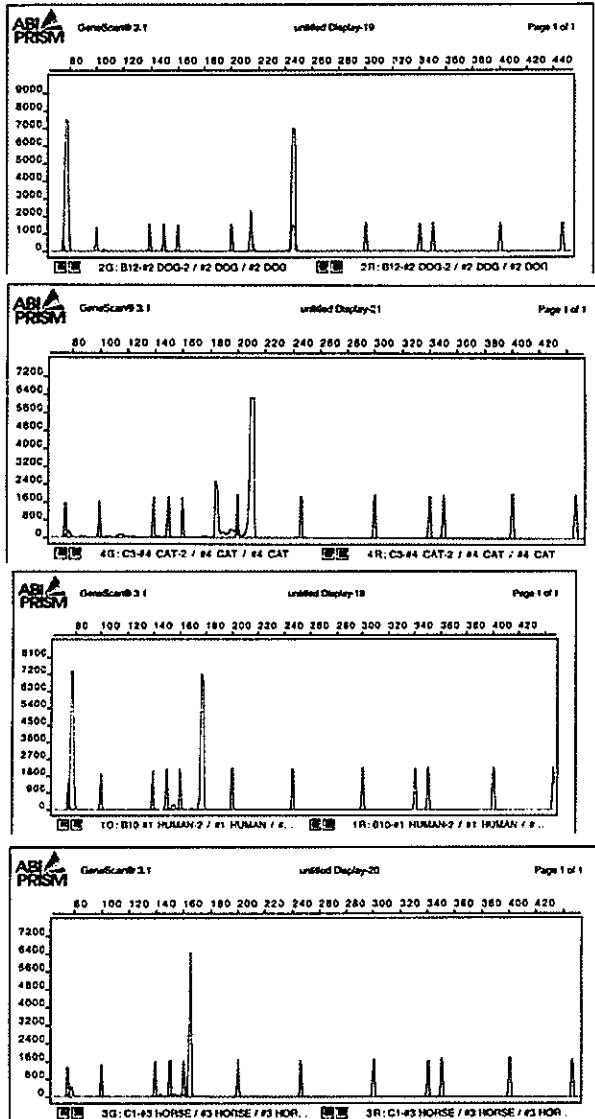


Figure 2. Single-plex results showing a composite of all four individual single-plex results from top to bottom: dog, cat, human, horse.

DNA-Based Species Testing

A PCR-based species can be devised based on mt Cox-I locus. The concept of doing species tests using amplification of Cox-I loci from human, horse, cat, and dog with JOE-labeled primers has been shown to work using gel separations. (Figure 1) In this work we established which variant of Taq polymerase would do best – and it was Taq Gold. The enzyme is robust, but requires a hot start (this is not a problem). As proof of concept, labeled PCR products from all the single-plexes and several multiplexes were run on the ABI 310. (Figure 2)

Although the final electropherograms show that there is still a need for refinement, we are satisfied that the concept we set out to test has been successfully demonstrated – namely, that a PCR-based species test can be devised, based on the mt Cox-I locus. Although four species were somewhat arbitrarily chosen for this proof of concept project, the technology could easily be extended to any species for which sequence information is available for the mt Cox-I locus.

The appeal of this approach is that it uses amplicon detection technology identical to that routinely employed in forensic labs for the detection of DNA profiling amplicon detection. Accordingly, the specimen preparation is very similar, and the software is set up to provide peak sizings. In practice, the use of an approach like this would entail running one extra tube per specimen of interest. Assuming there was no information from the case file to suggest non-human DNA, one could use a human-animal duplex, or human-animal-animal triplex, or even human-three other animal quadruplex. The expectation would be a human DNA amplicon but no animal ones. The result would then explicitly confirm human origin.

CONTACTS

R.E. Gaensslen
 Forensic Science Group
 Dept of Biopharmaceutical Sciences
 University of Illinois at Chicago
 833 S. Wood St., Chicago IL 60612
 (312) 996-2250
 reg@uic.edu

Barbara Llewellyn
 Illinois State Police
 Forensic Science Center at Chicago
 1941 W. Roosevelt Road
 Chicago IL 60608
 (312) 433-8000
 llewelb@isp.state.il.us

VALIDATION of Y-PLEX 12 DATABASE STUDY

FORENSIC TECHNOLOGY NEED

Forensic laboratories throughout the United States are grappling with how to manage an ever increasing backlog of forensic casework requiring DNA analysis and the costs associated with new methodologies in DNA analysis. DNA analyses conducted on evidence from criminal sexual assault cases are routinely used in forensic laboratories. The interpretation of DNA data on cases where more than one semen donor is detected is very difficult due to lack of automated techniques for the separation of female victim DNA from male sperm DNA. Y-Chromosome STR systems are used to resolve mixed DNA profiles in sexual assault cases.

The work was performed by Illinois State Police Forensic Science Command in collaboration with ReliaGene Technologies, Inc, multiplex Y-STR systems for use in forensic casework and in the development of a population database.

TECHNOLOGY DESCRIPTION

The first goal of this project is to evaluate and validate commercially available Y-STR systems which have the potential to resolve mixed DNA profiles in sexual assault cases. The second goal of this project is to generate a population database for Y-Chromosome STRs in the three major U.S. population categories: Caucasians, African-Americans, and Hispanics.

Y-Chromosome STRs are short tandem repeat loci on the Y-chromosome (found only in males) that have been found to exhibit detectable polymorphism. STR genotyping is performed by comparison of sample data to allelic ladders. In order to implement the use of Y-STRs in forensic casework, population data for the various loci need to be collected to

establish a population database. Because of the Y-Chromosome is inherited from father to son without recombination, there is less variability between individuals.

At the present time, the only statistical evaluation that can be conducted to give weight to a DNA match is the counting method. The statistical value obtained from the counting method is dependent on the number of samples that have been analyzed; therefore, it is imperative to have a large population database. These databases can only be generated by evaluating a large number of samples in three major categories: Caucasians, African-Americans, and Hispanics.

These Y-STR systems evaluations were conducted under the validation guidelines recommended by the Scientific Working Group on DNA Analysis Methods (SWGDAM). During the study, approximately 450 samples were collected and analyzed from the three major population categories utilizing Reliagene® Technologies Y-PLEX 12 amplification kit. Figure 1 shows an electropherogram example from the Y-PLEX 12 database population validation. The resulting profiles were added to an existing database maintained by Reliagene® Technologies.

TECHNOLOGY BENEFITS

- This Y-Plex 12 database study validated commercially available Y-STR systems for resolving mixed DNA profiles in sexual assault cases
- The inclusion of this additional data to the established Y-STR haplotype reference database will provide the

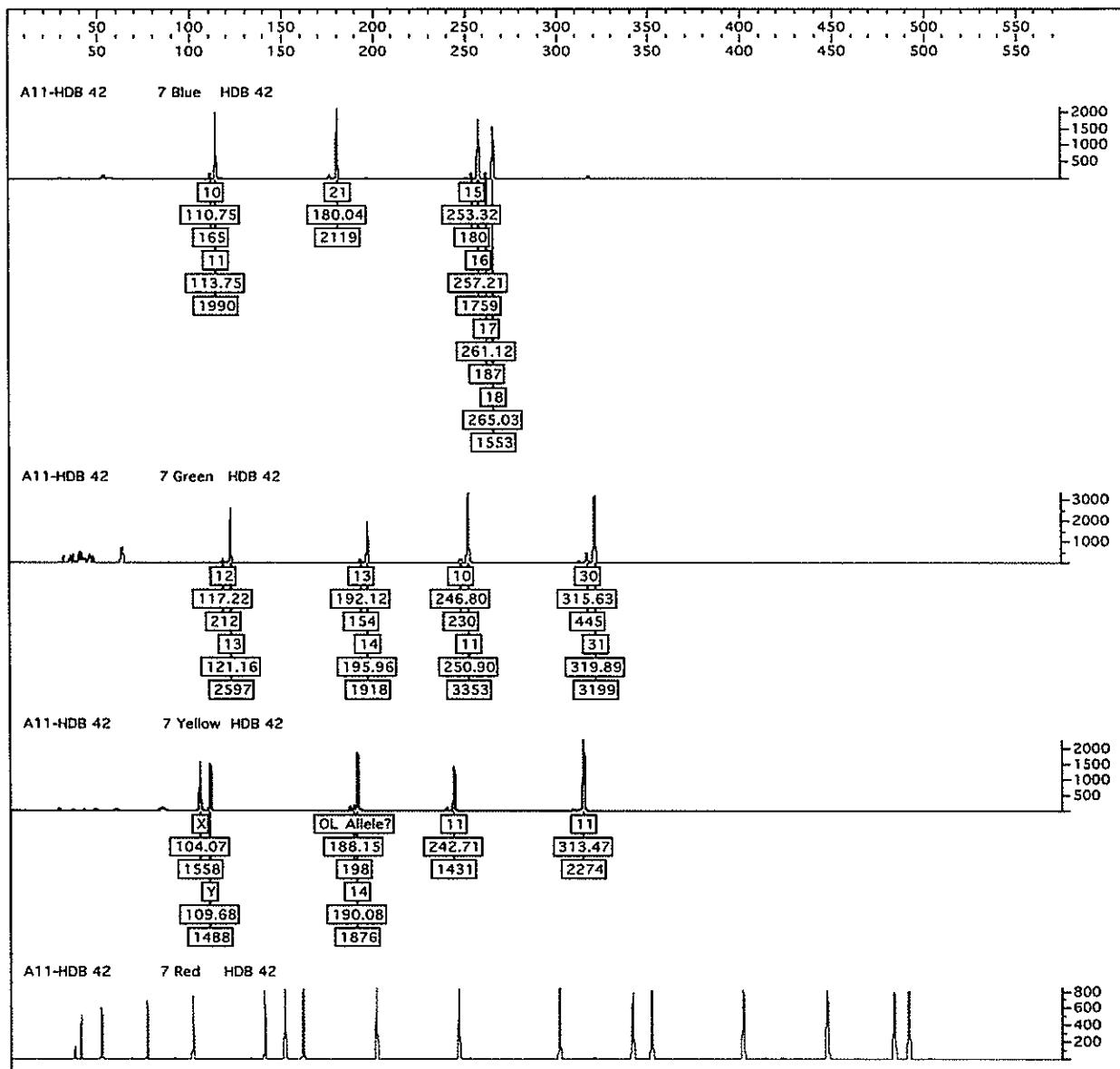


Figure 1. An example of an electropherogram from the Y-STR analysis showing microvariant sample alleles that can be compared to the allelic ladders for STR genotyping.

forensic science community with an additional analytical tool that will enhance DNA Services to the benefit of law enforcement and criminal justice.

- Forensic Laboratories can now utilize Y-STR analysis in forensic casework for the separation of female victim DNA from male sperm DNA

COLLABORATION/IMPLEMENTATION

The resulting profiles were added to an existing database maintained by Reliagene® Technologies. The inclusion of this additional data to the established Y-STR haplotype reference database provides forensic laboratories with an additional analytical DNA tool.

Publications and Presentations

- Frank, W.E., and S.N. Brown, "Validation of Y-PLEX 12 Database," *Midwest Forensics Resource Center/Ames Laboratory Final Report*, Ames, IA: Ames Laboratory, 2004, IS-5166.
- Frank, W.E., and S.N. Brown, "Validation of Y-PLEX 12 Database," Presentation at Midwest Forensics Resource Center Annual Meeting, July 15, 2004, Ames, IA, 50014.

ACCOMPLISHMENTS AND ONGOING WORK

This Y-Plex 12 database study generated the needed population data (Caucasians, African-Americans, and Hispanics) for the established Y-STR haplotype reference database. With this validated data, Forensic Laboratories can now utilize Y-STR analysis in forensic casework for the separation of female victim DNA from male sperm DNA.

Currently, Y-Plex 12 data is being collected and reviewed for Y-Plex Validation. Upon completion of this review, the results of the population database validation study will be submitted to the *Journal of Forensic Sciences*.

CONTACTS

Mr. William Frank
DNA Research Coordinator
Illinois State Police
Division of Forensic Services
Forensic Sciences Command
Research and Development Laboratory
2060 Hill Meadows Drive
Springfield, Illinois 62702
(217) 557-2399 fax: -3989
William_Frank@isp.state.il.us

Ms. Sandra N. Brown
Laboratory Director
Illinois State Police
Division of Forensic Services
Forensic Sciences Command
Research and Development Laboratory
2060 Hill Meadows Drive
Springfield, Illinois 62702
(217) 557-2399 fax: -3989
Sandy_Brown@isp.state.il.us

DEVELOPING APATAMERS TO METHAMPHETAMINE AS NUCLEIC ACID SENSORS

FORENSIC TECHNOLOGY NEED

The abuse of methamphetamine in the USA is described in a recent government publication by the director of the National Institute on Drug Abuse (NIDA) as "an extremely serious and growing problem". Methamphetamine traffic has increased significantly in the Midwest. A Department of Justice report states "an expert associated with Juvenile Court Services in Marshall County, Iowa, estimated in 1998 that one-third of the 1,600 students at Marshalltown High School had tried methamphetamine".

From 1994 to 1999 methamphetamine arrests rose almost 4-fold and meth lab seizures increased about 8-fold. Methamphetamine effects are long lasting and continue to cause damage long after the user has stopped taking the drug. Long-term effects can include fatal kidney and lung disorders, possible brain damage, depression, hallucinations, violent and aggressive behavior, weight loss, insomnia, behavior resembling paranoid schizophrenia, malnutrition, poor coping abilities, lowered resistance to illnesses, liver damage, stroke, and death. Societal impacts include crimes, fires due to explosions from the illegal manufacture of meth, and hazardous waste.

Aptamer-based assays will have several advantages over immunoassays for the rapid screening of biological samples. Chief amongst these advantages are that: 1) the aptamers are stable even when dehydrated and will be adaptable to mobile equipment, and 2) new aptamers can be rapidly "evolved" in the laboratory. Thus, the aptamer technology will be able to keep up with the introduction of new drugs and changes in drug use over time.

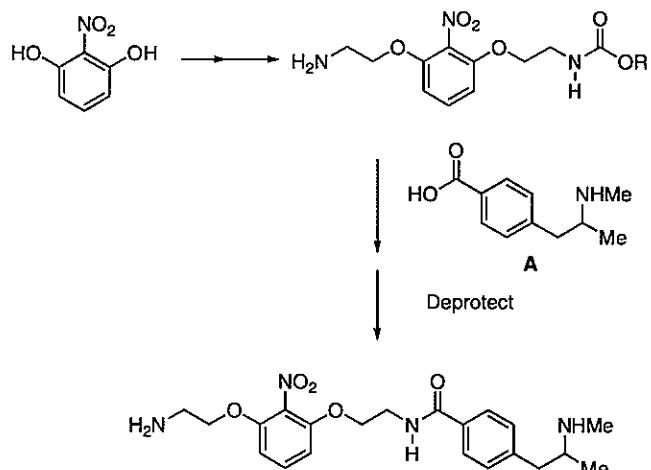


Figure 1. The meth analog **A** with a carboxylic acid tether that was prepared for this project. It has been connected to a UV active segment so that quantitation of the meth analog can be readily done.

TECHNOLOGY DESCRIPTION

The long-term goal of this project is to produce aptamers that can be used as screening tools for a variety of drugs to provide forensic investigators with rapid accurate screening procedures for common drugs. The following specific aims were proposed: 1) select for DNA aptamer(s) that recognizes methamphetamine 2) clone and characterize the isolated aptamer(s).

Aptamers are small nucleic acids that avidly and selectively bind small ligands with affinities in the nanomolar to picomolar range and with exquisite specificities. A column of methamphetamine-linked sepharose is used to select for the aptamer. To prepare the column, the compound can be prepared from the methyl ester of para-formylbenzoic acid and nitroethane and a primary amine in 78% yield (Kraus, unpub-lished). The crystalline product is then reduced in two steps to the amino ester which is hydrolyzed to the acid with lithium hydroxide (49% yield). The acid is mixed with

EAH Sepharose 4B (Pharmacia/Amersham) to selectively form a covalent linkage between the carboxylic acid group and the functional groups on the EAH Sepharose 4B which is designed to link with compounds containing free carboxyl groups in order to produce affinity resins (Figure 1).

The procedures for making single stranded DNA (ssDNA) aptamers are well described [1, 2]. Methamphetamine-linked magnetic beads are prepared for the SELEX procedure. A mixture of randomly varied ssDNAs is passed multiple times through a methamphetamine-agarose column with intervening amplification steps that also introduce new variations into the sequence. We began with a pool of single stranded DNA containing about 1014 random sequences. (Figure 2) Several rounds of SELEX are needed to select aptamers.

Methamphetamine is used to elute the methamphetamine-specific ssDNAs from the affinity column. After each round, the pool size of the DNA in the mixture will be monitored by determining the percent of total loaded ssDNA that is adsorbed and eluted from the affinity column. Negative selections against Sepharose and inactive methamphetamine

analogues are also included. The winning ssDNAs (that bind to the column) are cloned and sequenced to identify consensus sequences for aptamer structure. Consensus sequences for aptamers are synthesized chemically and tested for the ability to bind to methamphetamine-sepharose. The selected aptamers are also tested for their affinity to meth using a filter-based assay.

References

1. Tuerk, C. and L. Gold, "Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase." *Science* 249 [4968] (1990): 505-10.
2. Schneider, D.J., et al., "High-affinity ssDNA inhibitors of the reverse transcriptase of type 1 human immunodeficiency virus." *Biochemistry* 34[29] (1995): 9599-610.

TECHNOLOGY BENEFITS

Procedures, such as GC, HPLC, and MS that are currently used to analyze urine, hair, and other tissue samples for amphetamines and other drugs are costly and time consuming.

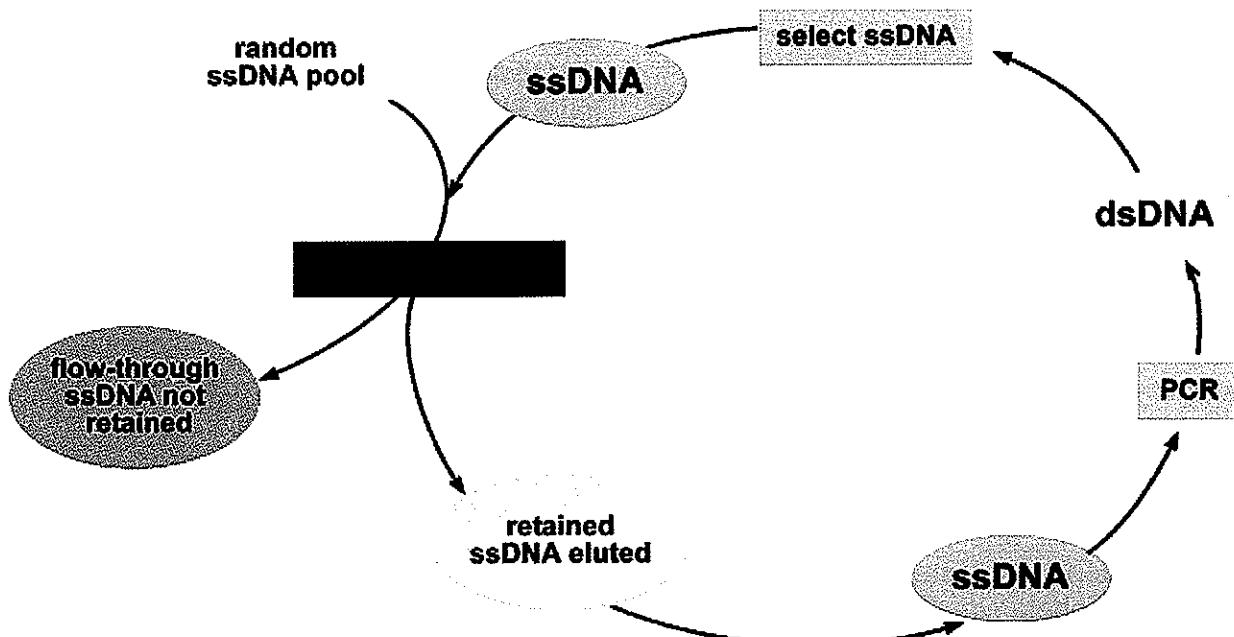


Figure 2. SELEX procedure used to optimize the first generation of aptamers.

Ideally, the initial screening for drugs, including methamphetamine, should be rapid and accurate.

ELISAs are currently being developed for initial screening because of their ease of performance and because these assays can be automated. Antibody microarrays are being developed to move the immuno-based methods of drug detection to a high-throughput, large-range assay. However, the obstacle remains that antibody responses are not constant between animals, suffer from batch-to-batch inconsistencies, and require that significant effort be dedicated to standardization.

Aptamers are nucleic acids with similar target-binding properties to antibodies. Unlike antibodies, aptamers are synthesized chemically and therefore have little batch to batch variation. Aptamers are ideally suited to use in analytical applications due to their small size and their stability to dehydration and high temperatures.

COLLABORATION

The results of the current proposed research will be disseminated by publication in research journals. The long-term goal of these studies is to develop aptamers that specifically recognize methamphetamine and other drugs and that can be used for the detection of these drugs at the crime scene and in the laboratory. We have contacted Sandy Stoltenow and Nila Bremer, scientists at the Iowa Division of Criminal Investigation and will work with them once we have obtained an aptamer that can be used to sense methamphetamine. Once established in Iowa, the use of aptamers for sensing drugs will be readily applicable to other states.

Publications and Presentations

- Kraus, G.A., and M. Nilsen-Hamilton. "Developing Aptamers to Methamphetamine as Nucleic Acid Sensors," Presentation at Midwest Forensics Resource Center Annual Meeting, July 15, 2004, Ames, IA, 50014.

ACCOMPLISHMENTS

We have now successfully passed through five rounds of positive selection in SELEX and one round of negative selection. Although a population has not yet evolved, we are carrying a subset of our oligonucleotides through these rounds and anticipate soon observing an evolving population. Frequently 12 to 20 rounds of SELEX are required to select aptamers. Once an aptamer population has evolved, the selected oligonucleotides will be cloned and consensus sequences identified for further development of the aptamers.

In addition to the SELEX, we also linked methamphetamine to a biphenyl diamine as a second methamphetamine derivative to use as target for the aptamers. By using this second target in later rounds, we eliminate from the pool potential aptamers that interact with the arm (resorcinol or biphenyl) that links the methamphetamine to the magnetic beads. The methamphetamine-(4-Bis (aminomethyl) biphenyl) will be used to prepare methamphetamine-linked magnetic beads for SELEX procedures.

CONTACTS

Marit Nilsen-Hamilton
Department of Biochemistry, Biophysics
and Molecular Biology
Iowa State University
3206 Molecular Biology Bldng
(515) 294-9996
marit@iastate.edu

George Kraus
Department of Chemistry
Iowa State University
2759 Gilman Hall
Ames, IA 50011
(515) 294-7794
gakraus@iastate.edu

LASER ABLATION-INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY OF FORENSIC GLASS SAMPLES

FORENSIC TECHNOLOGY NEED

Glass fragments are often recovered as trace evidence during criminal investigations. Because of the many different types of glasses manufactured, the challenge for the forensic examiner is to uniquely identify and characterize the glass trace evidence material from a crime scene with a high level of certainty that is defensible. Characterization of glass fragments is normally accomplished by measuring the physical and optical properties of density and refractive index. However, further discrimination, such as identification of a suspected source, has become more difficult as the range of refractive indices has narrowed within glass subtypes because of advances in glass manufacturing technology. Glasses from the same subtype, which have the same gross elemental composition, can have different trace and ultra-trace elemental signatures.

An obvious major source of this variation lies in the trace and ultra-trace elemental composition of raw materials consumed in the manufacturing process, which is dependent on where the raw materials are mined. The presence, absence, and relative abundance of elements in specific association patterns provides a unique means of display and comparison of the trace elemental signature for samples and is easily understandable. Therefore elemental compositional analysis has frequently been considered as an approach to classify glass fragments and differentiate between glasses within a class.

As a result, scientists have investigated the use of elemental analysis techniques, particularly inductively coupled plasma-atomic emission spectrometry and -mass spectrometry, for discrimination or differentiation between glasses within a

particular class (e.g., window glass) based on their trace elemental content. The implementation of laser ablation as a sampling technique extends this elemental analysis to much smaller sizes, typical of trace samples encountered in forensic cases.

TECHNOLOGY DESCRIPTION

This project involves the evaluation of laser ablation-inductively coupled plasma-mass spectrometry as an analysis technique that can differentiate glass fragments, which have similar refractive indices, based on the unique trace elemental signatures of the glass samples. Additionally, criteria and protocols for the comparison and differentiation of glass fragments from different sources, based on multivariate analysis techniques, have been developed. Multivariate analysis techniques allow use of the full acquired mass spectrum without any prior knowledge of the chemical composition of the sample.

A number of trace elemental analysis methods have been investigated for discrimination of glass samples. These methods include atomic absorption spectrometry, neutron activation, mass spectrometry, X-ray fluorescence, as well as inductively coupled plasma-atomic emission spectroscopy and -mass spectroscopy (ICP-AES and ICP-MS). Each method has its strengths, but only ICP methods have the precision, sensitivity, multi-element detection capability, and dynamic range suitable for trace elemental analysis of forensic glass samples. Although glass analysis methods by ICP-AES and ICP-MS are similar, ICP-MS has better sensitivity (by a factor of 100 for some elements) and has the capability to provide isotopic information for most elements.

TECHNOLOGY BENEFITS

ICP-MS provides a high level of discrimination for glass samples due to excellent detection limits (10-100 times better than AES), unparalleled elemental coverage, and isotopic information. Laser ablation-ICP-MS is rapid, eliminates the need for extensive sample preparation, and is a virtually nondestructive technique allowing the questioned samples to be further analyzed by corroborative techniques. Furthermore, laser ablation promises to increase the number of analytically useful elements detectable by standard ICP-MS techniques by eliminating problems with some elements due to poor dissolution and contamination. Additionally, smaller samples may be analyzed making the technique applicable to more cases.

A novel approach for sample comparison in this project is the utilization of multivariate analysis techniques, in particular Principle Component Analysis (PCA). PCA allows the use of the full mass spectrum without requiring any pre-selection or elimination of elements. PCA is a multivariate data reduction method that examines the variance patterns within a multidimensional dataset. PCA reduces the dimensionality of the dataset to a few simple variables while retaining a major portion of the mass spectral information. These new variables are then used for sample comparisons and derivation of statistical significance for the analysis.

COLLABORATION/ IMPLEMENTATION

The developed analysis protocols for glass analysis have been used for casework with the Vancouver, WA Police Department. Laser ablation-ICP-MS is a powerful and versatile technique for determining the elemental composition of a variety of different types of samples. The Materials Analysis Unit of the FBI laboratory has previously worked with the Ames Laboratory research group to transfer multivariate analysis methods developed for laser ablation-ICP-MS analysis of plain carbon steel. The analysis protocols were successfully implemented in a round robin organized by the Illinois State Police to

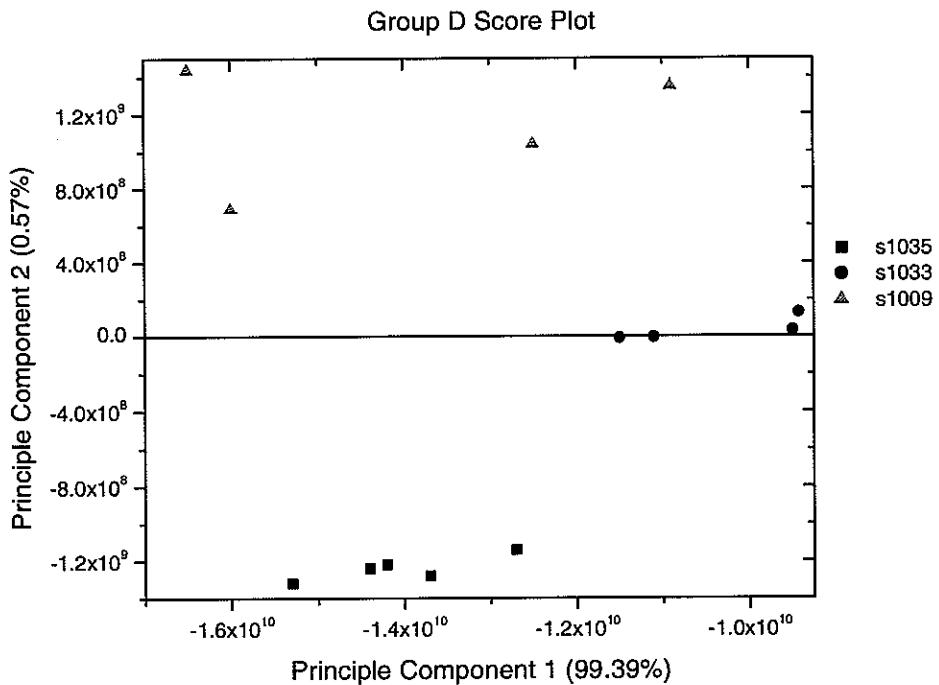
determine source attribution of glass fragments by different analytical techniques. The analysis protocols are currently being used in a collaborative study with Lawrence Berkley National Laboratory to investigate fractionation issues in nuclear proliferation materials. A manuscript outlining the experimental and analytical protocols for glass analysis is planned for submission to a forensic journal.

Publications and Presentations

- Baldwin, D.P., R.S. Houk, S.J. Bajic, "Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry of Forensic Glass Samples," *Midwest Forensics Resource Center/Ames Laboratory Final Report*. Ames, IA: Ames Laboratory, 2004, IS-5163.
- Bajic, S.J., D.P. Baldwin, D.B. Aeschliman, and R.S. Houk, "Laser Ablation Inductively Coupled Plasma Mass Spectrometry of Forensic Glass Samples," Oral Presentation at American Academy of Forensic Sciences, 56th Annual Meeting, 2004, Dallas, TX, Paper B164.
- Aeschliman, D.B., S.J. Bajic, D.P. Baldwin, and R.S. Houk, "Multivariate Pattern Matching of Trace Elements in Solids by Laser Ablation Inductively Coupled Plasma - Mass Spectrometry: Source Attribution and Diagnosis of Fractionation." *Analytical Chemistry* 76, (2004): 3119-3125.

ACCOMPLISHMENTS AND ONGOING WORK

This project determined that a minimum glass sample size of approximately 750 ng is required for an analysis by these methods. This amount of material would be completely consumed in an analysis. Work continues on understanding mechanisms that lead to fractionation and methods for addressing these issues. This work continues under US Department of Energy funding in collaboration with Lawrence Berkley National Laboratory. One area of investigation includes the use of ultrafast lasers to eliminate fractionation.



This plot illustrates the Principle Component Analysis (PCA) of the mass spectra acquired by LA-ICP-MS from three glass fragments that are not differentiable based on their refractive indices or density. The entire mass spectra (elemental signatures of the samples) are used in the analysis. PCA reduces the mass spectra to a few variables, which are used for sample comparisons. When these variables are plotted, the repetitions of the samples cluster together, but the samples occupy different areas on the plot indicating that the glass samples are distinguishable based on their elemental compositions.

CONTACTS

Dr. David P. Baldwin
 Ames Laboratory
 130 Spedding Hall
 Ames, IA 50011
 (515) 294-2069 fax: -4748
 dbaldwin@ameslab.gov

Dr. R. Sam Houk
 Ames Laboratory
 B27 Spedding Hall
 Ames, IA 50011
 (515) 294-9462 fax: -5233
 rshouk@iastate.edu

Dr. Stanley J. Bajic
 Ames Laboratory
 9 Spedding Hall
 Ames, IA 50011
 (515) 294-2086 fax: -4748
 sjbajic@ameslab.gov

FORENSIC ANALYSIS OF TRACE EXPLOSIVES

FORENSIC TECHNOLOGY NEED

The rise in terrorist bombings has made the chemical identification of explosives a priority for the forensic scientist. The volume of evidence to be analyzed and the importance of such testing for preventative and investigative purposes have increased the need for detection methods specific for explosives that are reliable, fast, and cost effective. In this project, work was performed, as a collaboration between the Chemistry Department at the University of Nebraska-Lincoln and the Nebraska State Patrol Crime Laboratory, to develop new analytical methods for the forensic analysis of trace explosives by combining immunoextraction and capillary electrophoresis (CE).

TECHNOLOGY DESCRIPTION

The analysis method created in this project for trace explosives makes use of antibody-based extraction (i.e., immunoextraction) for the concentration and selective isolation of desired explosive agents, followed by the separation and analysis of the isolated agents by CE (Figure 1). The immunoextraction sample pre-treatment and CE portions were developed separately and then merged to create a tandem analytical system.

High-performance liquid chromatography (HPLC) and gas chromatography (GC) are two common methods employed in analyzing explosives. CE is an alternative that has been explored for detecting explosives. Some of the advantages of CE versus HPLC include smaller sample volume requirements and greater efficiency, allowing CE to separate more compounds per run. CE, in contrast to GC, can work directly with liquid samples and uses lower separation temperatures, which is important when dealing with thermally unstable explosive compounds. Both HPLC and GC

are used for moderate to large amounts of sample. The CE method developed here utilizes nanoliter amounts of sample, unlike the milliliter sample amounts required in HPLC, and in a nondestructive manner (unlike GC). In addition, this CE method requires only nanoliter amounts of other reagents.

TECHNOLOGY BENEFITS

The CE method developed in this project has been developed to separate fourteen target organic compounds including HMX, RDX, TNT, PETN, and Tetralyl. The current CE protocol has brought the following benefits:

- Nanoliter sample requirements, translating into lower consumption of evidence
- 15-minute total analysis time per sample
- Smaller reagent requirements than currently employed methods lowers purchasing and waste disposal cost

In addition, immunoextraction columns have been successfully developed and tested for both HMX and TNT-related compounds. The binding and elution properties of these columns have been determined, as well as some of the cross-reactivity with related agents. Based on past work that has been performed with similar columns for herbicides and pesticide analysis in environmental samples, the availability of such columns should greatly reduce the amount of time and effort that is required by forensic scientists to process explosives samples prior to analysis.

COLLABORATION/ IMPLEMENTATION

The Nebraska State Patrol Criminalistics Laboratory has provided valuable guidance in the development of these methods. This has helped ensure that the protocols and instrumentation that have been developed are

Figure 1. Separation & Analysis of Several Explosive Agents & Related Compounds by CE

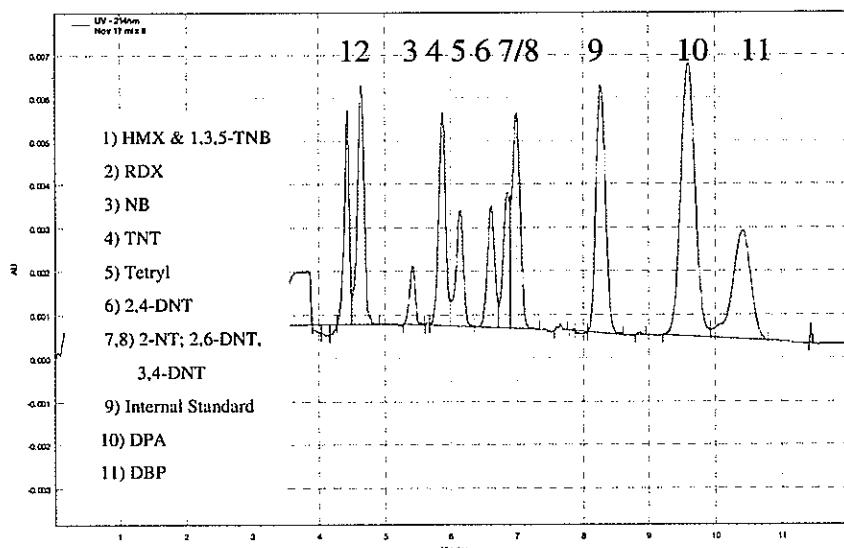


Figure 1. Separation and analysis of several explosive agents and related compounds by capillary electrophoresis.

practical and relevant to the forensic science field. Dissemination of this research has been conducted through presentations that have been made at the 2004 Joint Meeting of the Midwest, Mid-Atlantic, Southern, and Canadian Societies of Forensic Scientists. An invited presentation was also made on this work at a special meeting that was held in Fall 2004 by the U.S. Army Corps of Engineers on Biosensing Methods for Explosives. In addition, the PI's laboratory is planning to submit a paper on this topic for publication in the *Journal of Forensic Sciences* by May 2005.

The PI's laboratory is continuing to focus on this project with work being done to further optimize the CE separation and immunoextraction. Future work will focus on miniaturizing the analytical system used in this project for the purpose of developing a portable explosives detection system for screening evidence at the site of an explosion.

The Army Corps of Engineers has contacted the PI with an interest in funding field studies with this approach, which are scheduled to take place in the summer of 2005.

Publications and Presentations

- Hage, D.S., "Forensic Analysis of Trace Explosives," *Midwest Forensics Resource Center/Ames Laboratory Final Report*, Ames, IA: Ames Laboratory, 2005, IS-5168.
- Burks, R. "Forensic Analysis of Trace Explosives," Presentation at Midwest Forensics Resource Center Annual Meeting, July 15, 2004, Ames, IA, 50014.

CONTACTS

Principal Investigator:

David S. Hage
Chemistry Dept., Univ. Nebraska
608 Hamilton Hall, Lincoln NE 68588
(402)-472-2744 fax: -9402
dhage@unlserve.unl.edu

Technology User:

Dr. John Dietrich
Nebraska State Patrol Crime Laboratory
1223 Arapahoe, Lincoln, NE 68502
(402)-471-8950
jdietrich@nsp.state.ne.us

