Forensic Evidence

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September 2001
Volume 49
Number 5

United States
Department of Justice
Executive Office for
United States Attorneys
Office of Legal Education
Washington, DC
20535

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Director

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The United States Attorneys' Bulletin is published pursuant to 28 CFR § 0.22(b)

The United States Attorneys' Bulletin is published bi-monthly by the Executive Office for United States Attorneys, Office of Legal Education, 1620 Pendleton Street, Columbia, South Carolina 29201. Periodical postage paid at Washington, D.C. Postmaster: Send address changes to Editor, United States Attorneys' Bulletin, Office of Legal Education, 1620 Pendleton Street, Columbia, South Carolina 29201

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Trace Evidence - Hairs/Fibers - Crime Scene

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I. Trace Evidence

Evidence that is generally small in size and is often easily transferred is commonly referred to as "trace evidence". "Trace evidence" can take the form of fibers, paint chips, soil, building materials, glass, gun shot residue, seeds, feathers, animal hair, human hair, wood fragments and other materials. These substances are often exchanged between individuals during physical contact. They can also be transferred from individuals to environments and from environments to individuals. They can define where an individual may have been, with whom the contact occurred, and perhaps, the nature of the contact. Trace evidence provides significant pieces to the puzzle of a violent crime.

When does trace evidence become an issue in a criminal investigation? Many times it occurs well after the commission of the crime, well after the charges have been filed, and well after the completion of forensic examinations. It often occurs when a prosecutor is evaluating the evidence prior to trial, and questions why certain types of trace materials were never examined. At this stage, it may be too late to conduct meaningful trace examinations due to possible contamination at the scene or in the laboratory.

II. The Crime Scene

Trace evidence transfers occur all the time, and great caution should be used in the examination of a crime scene. Care should be taken to minimize the extent of additional transfers that occur once investigators are at the scene. Crime scene investigators will often wear special clothing such as coveralls, hair bonnets and booties to help prevent additional fibers and hairs from being added to the scene.

Care should also be taken when assigning individuals to related crime scenes. There is a perceived risk when one individual collects evidence from the suspect's residence and, later the same day, collects evidence from the victim's residence. Even though precautions may have been taken by that individual, the fact that the same person collected evidence from both locations can have a negative impact on the value of forensic tests derived from that evidence.

The manner in which a crime scene is searched is determined by the type of crime, the location of the scene, details concerning events of the crime, the time of day, the number of people available for the search and equipment. Inasmuch as hair and fiber evidence can play a role in most cases involving violent crime, serious consideration should be given to collecting it properly. Once the crime has been committed, it won't be long before hair and fiber evidence will be lost or contaminated. The importance of securing the crime scene cannot be overstated.

When physical contact occurs between individuals, objects and individuals, or two objects, there is a likelihood of a transfer of hair and fiber evidence. This likelihood is dependent on the nature and duration of the contact as well as the nature of the contacting surfaces. The direct transfer of hairs from the head of an individual to the clothing of another individual is called a primary transfer. When hairs have already been shed and are transferred to an individual, it is called secondary transfer. Fibers are transferred in a similar manner. When fibers are transferred from the fabric of an individual's clothing to the clothing of another individual, it is called a primary transfer. As these same fibers are transferred to other objects during subsequent contacts, secondary transfers are occurring.

It is important for crime scene investigators to understand the mechanisms of primary and

secondary transfer. As trace evidence can be transferred during the commission of a crime, it can also be transferred during the search process. Hairs and fibers can not only be picked up inadvertently by investigators, they can be inadvertently deposited at the crime scene. The following are considerations at the crime scene:

a. Know the personnel conducting the search - you may need to obtain elimination hair and/or fiber samples.

b. Prioritize the order of evidence collection. Collect large items first and then proceed to the trace evidence — WATCH WHERE YOU STEP!

c. Once the trace evidence is collected (vacuuming/taping/tweezing) you can proceed to take samples of blood, remove bullets from walls and dust for fingerprints.

d. Processing the crime scene for fingerprints prior to trace evidence collection is not recommended, as the fingerprinting process may:

   1. Inadvertently remove trace evidence onto the clothing of the technicians;

   2. Move trace evidence;

   3. Contaminate hair and fiber evidence with dusting powder that is difficult to remove.

The following are suggestions for collecting evidence from a crime scene such as a house, apartment or automobile:

a. Photograph the scene prior to removing evidence.

b. Remove larger items/debris from the carpeting or walk areas prior to other examinations. Disposable "booties" should be worn and collected later.

c. Collect large items such as clothing and place them in brown paper bags. Keep an accurate evidence log. Have one person collect the items and place them in bags, while the other person records the items and labels the bags.

d. Do not place all clothing items from a suspect in one bag. Likewise, do not place all items from a victim in a single bag.

e. Never put suspect items and victim items in contact with one another. The person collecting the suspect's items should not collect the victim's items. If this must occur, be sure to change clothing between collections and do them at different times to avoid contamination.

f. Bedding should be carefully handled to avoid loss of hairs and fibers. Each item should be placed in a separate bag.

g. Floor surfaces should be vacuumed for possible trace evidence. Some crime scene investigators may use tape to secure trace evidence. This is generally difficult to work with, both at the scene and in the laboratory. However, smaller surfaces such as chairs, car seats, etc. can be taped or vacuumed.

h. Make sure carpet standards, pet hair samples and other standards that realistically might have transferred to a suspect or victim are collected.

i. Always process for fingerprints after collecting trace evidence.

j. In a vehicle, be sure to collect all possible "known" fiber samples. These may be obtained from the carpet, door panels, headliner, seats, floor mats, trunk, etc.

The following suggestions pertain to different types of items recovered at the crime scene:

Hats:

Secure all hats in separate bags. Be careful when collecting baseball style caps with adjustable plastic head bands — these bands are an excellent source for fingerprints. Knit hats should be packaged as they were found.

Shoes:

Shoes are an excellent source of fiber evidence, blood stains, shoe print comparisons, etc. Shoes worn by a suspect can deposit fibers from the vehicle used at a crime scene and can also pick up fibers from the scene and deposit them in another location.

Socks:

Socks worn by a homicide victim, e.g. laying along a roadside, can provide invaluable fiber and hair evidence. Many times the victim is driven to an isolated area in a car or van. Contact with the interior surfaces of a vehicle can cause hairs and fibers to collect on the socks. It may be necessary to obtain elimination samples of the carpeting of the victim's car or residence to avoid the possibility of a coincidental match.

Fingernails:

Care should be taken when scraping or clipping the fingernails of a victim/suspect. The smallest amount of DNA on the hands or utensils of the medical personnel can contaminate the material and influence the DNA results.

Hairs in the hands of the victim:

Generally hairs found in the hands of the victim come from the victim. Rarely do the hairs belong to the suspect. Still, these must be collected and submitted for analysis.

Pubic/head hair combings:

These samples should always be taken in violent crimes. Foreign hairs, as well as fibers, can be recovered from these samples. If a hat is recovered at the crime scene and a suspect is identified soon, it may be possible to find fibers from the hat in the suspect's hair.

Weapons:

Weapons recovered at a crime scene should always be checked for the presence of trace evidence before processing for fingerprints.

Doors/windows:

These should be checked for hair and fiber evidence if they are points of entry or exit.

Known hair samples:

Good, thorough, random samples should be taken from the head/pubic regions of the suspect(s) and victim(s). Twenty-five, full-length hairs, pulled and combed from different areas of the head and pubic regions are generally considered adequate to represent an individual's hair characteristics.

III. Evidence Handling Procedures

Once the evidence has been collected, there are several recommendations or considerations when packaging it for transmittal to the laboratory. Crime scene items may include clothing worn by the suspect and victim, bedding, and known hair samples. It is important that the individual clothing items be packaged in separate, sealed paper bags — not plastic. All damp or blood-soaked items must be air-dried in a room away from air movement and traffic. To avoid contamination, clothing items from the suspect should never be handled in the same area where items from the victim are handled. Drying paper placed under damp clothing items should be submitted separately.

Individual hairs and fibers should be placed in a druggist fold in a sealed envelope (all corners must be taped). Individual hairs identified on items of clothing are often not removed or secured. These hairs may move or be lost, so it is recommended that they be removed and placed in an envelope (first noting where they were removed).

If a floor surface is vacuumed, the debris should be placed on a white sheet of paper (8" X 11") and made into a druggist fold. Then place the druggist fold in a clear zip-lock bag.

IV. Routine Protocol for Evidence Processing in the FBI Lab

When evidence is received in the FBI Laboratory, the case is assigned to an examiner.

The examiner will read the incoming communication to determine the nature of the offense, the names of the suspects and victims, and the types of examinations requested. It is important to have an understanding of the offense to help determine the course of action in the laboratory. For example, it would be important to know the relationship of the suspect and victim which might influence the significance of trace evidence collected from these individuals.

The laboratory unit has three processing rooms that are designed for debris collection. This arrangement allows for the processing of suspect clothing, victim clothing, and crime scene evidence in different rooms. Debris is collected through a combination of picking, scraping and/or taping. Vacuuming is not recommended for clothing items. The debris that is collected by scraping is placed in pillboxes and tapings are secured in clear plastic document sleeves. Pillboxes and tape strips are examined with a stereobinocular microscope using incident and transmitted light. The hairs and fibers are mounted on glass microscope slides for identification and comparison purposes.

V. Conclusions

When a questioned hair exhibits the same microscopic characteristics as the known hairs of an individual, the hair *could* have originated from that individual. If the questioned hair is microscopically dissimilar to the known hair standard, it cannot be associated to the individual. Differences in microscopic characteristics can be the result of time and alteration. Known hair samples should be collected from individuals as soon as possible after the date of the crime. As time passes, microscopic characteristics can change or the individual may alter the color with dyes. It is important to know that different people generally have different hair characteristics.

Nuclear and mitochondrial DNA analyses are meaningful adjunct examinations to microscopical comparisons. While the microscopic comparison of human hairs is a very useful technique, DNA technologies can resolve identity issues when the questioned and known hair samples exhibit the same microscopic characteristics.

If a textile fiber exhibits the same microscopic and optical properties as a known fabric, the fiber *could* have originated from that fabric. It is not possible to say that a fiber *originated* from a particular fabric. However, textile fiber associations are not insignificant. Because of the many different types of fibers and fabrics and the many different ways they can be colored and processed, the likelihood of finding coincidental fiber associations is remote.❖

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Another Powerful Forensic Genetic Tool: Mitochondrial DNA Typing

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I. Introduction

Touted as the most significant analytical tool introduced into forensic science since fingerprinting, DNA typing of biological evidence has far exceeded expectations. Because of its sensitivity of detection and genetic resolving power, DNA typing enables exculpation of those individuals falsely associated with evidence, potentially provides solid identification of donors of evidence, potentially enables identification of remains of missing persons, and can elucidate family relationships. However, the technology's impact has been more far-reaching. There are now, in forensic science, effective quality assurance standards, proficiency testing requirements, interpretation guidelines, training guidelines, requirements for higher education and continuing education, national felon DNA databanks, and even changes in legislation, such as post-conviction analyses. Moreover, DNA typing has become routine in cases where biological evidence may be meaningful.

DNA can be considered a genetic blueprint of an individual. The complete blueprint can be found in each nucleated cell of a person's body and is constant throughout the life of an individual. DNA found in the nucleus of a cell is termed "nuclear DNA." The nuclear DNA is divided among and packaged into twenty-three different chromosomes in an individual (males have an X and a Y chromosome and therefore technically have twenty-four different chromosomes; but there are twenty-three chromosomes to a set of human DNA). There are two sets of twenty-three chromosomes in each cell (excluding sperm and eggs which only have one set of chromosomes); one set is inherited from an individual's mother and the other set is inherited from the individual's father. Thus, most genetic markers exist as two copies in each nucleus (Figure 1). The different forms of a genetic marker are called alleles.

Generally, any biological material that contains nucleated cells, including blood, semen, saliva, hair, bones, and teeth, potentially can be typed for nuclear DNA markers (or genetic polymorphisms). The technology available today includes a myriad of genetic markers, a variety of valid DNA typing strategies, and computers with specialized software. The methods used routinely for human identity testing include restriction fragment length polymorphism (RFLP) typing of variable number of tandem repeat (VNTR) loci (9,

21, 34, 35, 69, 78), and amplification of target DNA molecules by the polymerase chain reaction (PCR) (65) with subsequent typing of specified genetic markers (10, 11, 12, 16, 18, 36, 64, 76, 77). These nuclear DNA typing methods and their applications have been well-described in the scientific literature and, thus, will not be discussed further in this article. Instead, the focus of this paper will be on another piece of genetic material found within the cell, but outside the nucleus - mitochondrial DNA (mtDNA).

II. Mitochondrial DNA

Typing mtDNA affords greater sensitivity of detection which enables analysis of severely degraded materials. MtDNA analysis is particularly useful for genetic typing of bones, teeth and hair, and elucidating some family relationships (1, 6, 20, 22, 29, 30, 33, 39, 42, 45, 63, 67, 71, 76, 77).

Mitochondria, known as the powerhouses of the cell, are subcellular organelles that contain their own chromosome that is separate and distinct from the nuclear DNA chromosomes (Figure 1). Human mtDNA differs from nuclear DNA in that it is a circular piece of DNA, it is inherited solely from the mother, and it occurs in hundreds to thousands of copies per cell.

Some regions of the mtDNA chromosome have a high degree of variation among the human population. The highest degree of variation in the mtDNA chromosome among individuals is found within the non-coding region. Two areas within the non-coding region termed hypervariable regions I and II (HVI and HVII, respectively) are typically sequenced (2, 13, 73) (Figure 2). Excluding mutations, a mtDNA sequence is identical for all maternally related relatives (14, 19, 32, 62). In general, the transmission of a mtDNA type is consistent across generations. This feature of maternal inheritance can be useful in establishing, or refuting, identity of putative samples by using known maternal relatives as reference material to compare with the questioned mtDNA type (20, 22, 30, 33, 45, 71). Thus, unlike nuclear genetic markers, relationships several generations removed may be evaluated by mtDNA typing (Figure 3).

Generally, only one type of mtDNA sequence should be observed per individual. If only one mtDNA type is observed, the individual is considered to be homoplasmic. However, a condition known as heteroplasmy can occur (3, 4, 15, 16, 23, 28, 70, 75). Heteroplasmy is defined as more than one mtDNA type being carried by an individual. The different types operationally observed in an individual usually differ at only one genetic site in the sequence. Heteroplasmy may be observed in several ways: 1) individuals may have more than one mtDNA type in a single tissue; 2) individuals may exhibit one mtDNA type in one tissue and a slightly different type in another tissue; and/or 3) individuals may be hemoplasmic in one tissue sample and homoplasmic in another tissue sample.

While there are different characteristics of mtDNA compared with nuclear DNA, the forensic application of mtDNA typing is basically similar to nuclear DNA, or other forensic tests, and is nothing more than a pattern comparison. A mtDNA pattern (or profile or sequence) is generated from an evidence sample, and it is compared with a mtDNA sequence derived from a reference sample. If the two sequences are sufficiently different, then the two samples could not have originated from the same source. However, if the two sequences are sufficiently similar, then they cannot be excluded as originating from the same source. If a failure to exclude is obtained, inferences can be made, through statistics, to convey the significance of the match.

Sequence analysis of human mtDNA extracted from forensic biological specimens has been available as a routine forensic tool since the mid 1990's (1, 6, 20, 22, 29, 30, 33, 39, 42, 45, 76, 77) and has culminated in the implementation of the FBI's National Missing Person DNA Database (NMPDD). Hair shafts, bones, teeth and other samples that are severely decomposed and may not be typeable with nuclear DNA methods, may be characterized with mtDNA due to the high

copy number of mtDNA (5). For example, 7000-year-old brain tissue (57), 5,500-year-old bone (59), 4000-year-old mummified tissue (58), the remains of a Neanderthal man (39), and bones from American war casualties have been mtDNA-sequenced successfully (30). A well known example of mtDNA analysis for identity purposes is the verification of bones originating from Tsar Nicholas II. Using mtDNA obtained from living maternal relatives (Countess Xenia Cheremeteff-Sfiri and the Duke of Edinburgh), a comparison was made with the sequence of mtDNA extracted from putative bones of the Tsar. The sequences were similar, and the data supported the hypothesis that the putative remains were those of Tsar Nicholas II (20, 33).

Another important application of mtDNA sequencing in forensics is the potential analysis of hair shafts. Since single hair shafts contain too small a quantity of nuclear DNA, mtDNA sequence analysis may be the only viable technique for analysis. In fact, sequences can be obtained from as little as one to two centimeters of a single hair shaft (29, 42, 73, 76, 77).

III. Typing Methodology

The double-stranded DNA molecule has a shape similar to that of a spiral staircase. If stretched out, the double-stranded molecule looks like a railroad track. The rails, or strands, are each a polymer (i.e., a long chain) composed of four building blocks called nucleotides or bases (designated A, C, G, or T). The sequence of the different bases can be in any order along this polynucleotide chain. An enormous array of different sequences can be generated with the four different nucleotides within a relatively short DNA fragment. The two strands associate by chemical bonds between bases on each strand (these bonds would be the wooden slats connecting the two rails of the railroad track). An A on one strand will only bond with a T on the other strand. Similarly, G and C on opposite strands can bond. Thus, if the sequence of one DNA strand is known, for example AAGCTAC, then the complementary strand sequence can be deduced, i.e, in this case TTCGATG (Figure 4). It is this phenomenon of complementary binding that is exploited in all DNA typing methods. When in the single stranded state and under appropriate analytical conditions, a DNA molecule will bind only to its complement.

To prepare the DNA from forensic samples for mtDNA sequencing (see Figure 5a for steps to mtDNA analysis), the polymerase chain reaction (PCR) is employed (65). PCR is an *in vitro* process that can be thought of as a form of "molecular Xeroxing." The salient feature of PCR is the ability to obtain relatively large amounts of specific DNA sequences from relatively small quantities of DNA (Figure 5b). The PCR is a particularly useful tool for the analysis of forensic material (which may be somewhat degraded). In fact, minute quantities of DNA extracted from the following materials are typed routinely and successfully using PCR-based assays in forensic laboratories: 1) blood (i.e., lymphocytes), semen (i.e., sperm and to a lesser degree lymphocytes), saliva (i.e., epithelial cells), and sweat (i.e., skin cells) deposited on various substrates including clothing, cigarettes, postage stamps, envelope flaps, drinking straws and containers, chewing gum, and face masks; 2) vaginal swabs from rape victims; 3) various tissues from human remains; 4) personal items, such as hair brushes, tooth brushes, and razors which may provide a source of reference samples for identification of unknown remains.

After the PCR amplifies a sufficient quantity of the hypervariable regions of the mtDNA, the sequence of the nucleotides (i.e., genetic letters to the DNA code A, G, C, and T) can be determined in the mtDNA (Figure 5c). The reagents required to perform sequencing (i.e., the Sanger sequencing method (66)) are standard reagents and include: purified, single-stranded DNA template (which is obtained from the PCR-amplified sample), a DNA primer (a short piece of DNA to initiate the sequencing reaction), the four DNA building blocks (A, C, G, and T - collectively known as dNTPs), four specialized building blocks of terminator analogs that halt or terminate DNA synthesis (collectively known as ddNTPs), and a DNA polymerase (an enzyme that

enables synthesis of DNA by extending the primer and adding DNA building blocks in an order dictated by the amplified DNA template).

Basically, the PCR generates sufficient DNA template for sequencing. The double- stranded DNA template is split into single strands and then the sequencing primer is allowed to bind to one of the single-stranded DNA molecules. The primer is extended, by action of the DNA enzyme, across the target DNA by the sequential addition of the four dNTPs and, eventually, one of the terminator ddNTPs. When a ddNTP is incorporated into the growing chain by complementary base pairing to the template, chain elongation is terminated at the point where the ddNTP is incorporated. The particular ddNTP that is incorporated (i.e., A, G, C, or T) indicates that genetic letter and its position in the sequence. By reading the sequence electropherogram (Figure 6), the sequence of the sample can be deduced.

IV. Mitochondrial DNA Nomenclature

The first entire human mtDNA sequence was described by Anderson, et al. (2), and this sequence is used as a reference standard to facilitate nomenclature of mtDNA types. When a difference in an individual's sequence compared with that of the Anderson, et al. (2) sequence is observed (known as a polymorphism with respect to the Anderson or Cambridge Reference sequence), only the site (which has a designated number) and the nucleotide differing from the reference standard are recorded. For example, at site 263 (in HVII), the Cambridge Reference sequence has an A; however, a person may carry a G at site 263. Such an individual's mtDNA sequence is described as 263G. If no other bases (or sites) are described, then it is understood that the particular mtDNA sequence is identical to the Cambridge Reference sequence, except as noted at site 263. If an unresolved ambiguity is observed at any site, the base number for the site is listed followed by an "N" (e.g., 16228N). An "N" designation is essentially a "wild card" in that any base (A, G, C, or T) can be compared and considered equivalent for matching purposes. An insertion (an additional base in the sequence compared with the Cambridge Reference Sequence) is described by first noting the site immediately to the left of the insertion followed by a point and a "1" (for the first insertion), a "2" (if there is a second insertion), and so on, and then by the nucleotide that is inserted. For example, a common insertion is 315.1C. This polymorphism occurs after site 315 where a C is inserted. Deletions are recorded by listing the missing site followed by a "-" (i.e., 249-).

V. Interpretation

Interpreting mtDNA results is fairly straightforward. Typically sequence concordance is assessed between reference and evidence mtDNA sequences. Concordance occurs when the reference and evidence samples share a common DNA sequence. If the sequence in the evidence and that in the reference sample are identical at every compared position in the sequence, they are considered concordant, and it may be concluded there is a failure to exclude these samples as possibly originating from the same source (Figure 7a). If the two compared sequences are sufficiently dissimilar, then the samples can be considered to have originated from different sources (Figure 7b). When heteroplasmy arises, careful analysis and direct comparisons between multiple reference samples and a questioned sample should, in most cases, alleviate interpretational differences. If the mtDNA sequences from two samples being compared demonstrate the heteroplasmy (i.e., both sequences are observed in each sample), the interpretation is "cannot exclude" (or they are concordant). If they share a common sequence (i.e., one sample is heteroplasmic and the other homoplasmic, and one of the heteroplasmic types is concordant with the homoplasmic type), then the interpretation is a "failure to exclude" (Figure 8b). If both samples are deemed homoplasmic and differ slightly (i.e., typically at only one site), further investigation is warranted, such as typing additional reference samples. If no resolution can be attained, the interpretation is "inconclusive" (i.e., there is insufficient information to render a conclusion).

When a mtDNA sequence from an evidence sample and one from a known reference sample cannot be excluded as originating from the same source, it is desirable to convey some information about the rarity of the mtDNA profile. The current practice is to count the number of times a particular sequence is observed in a population database(s). Because of the uncertainty involved in all population database samplings, a confidence interval can be placed on the observation. A confidence interval is a measure of the amount of confidence which can be placed on a value lying between two specified limits (i.e., the interval). The use of a confidence interval around the counted number of mtDNA sequences in a database sample is similar to the method for placing a range on polling estimates. Thus, based on the size of the database(s), a range of uncertainty is placed on the frequency of a mtDNA type. However, only the upper (or conservative) bound estimate is provided to the fact finder. For example, consider a mtDNA sequence obtained from an evidentiary sample that is compared to a database containing 500 typed samples to evaluate the significance (or weight) of the evidence, and no matching sequences were observed. Thus, there were zero observations in the database of five hundred people. The 0/500 value is the counting method approach and is typically presented. However, when requested (to account for possible sampling error), the upper bound estimate can be calculated and, in this particular case, that value would be 0.6% of the population that could possibly carry the type. On the other hand, if five people in the database were to have the same mtDNA sequence as the evidence sample, the counting method estimate would be five out of 500 (or 1.0%). However, the range in this case would be from 0.6% to 1.4%, but only the 1.4% value would be presented.

VI. Databases

According to the FBI's National Center for the Analysis of Violent Crime, between one hundred fifty and two hundred children are involved in long term, non-familial abductions each year. Many of these missing children are never located. In any given calendar year, the skeletal remains of over one hundred unidentified individuals are located throughout the United States (personal communication, John E.B. Stewart, FBI, NMPDD). When an individual or the skeletal remains of an individual cannot be identified by fingerprint, dental, medical, or anthropological examinations, DNA from relatives of the missing person can be compared to DNA from the person or the remains of a person for identification purposes. A database that stores the known mtDNA sequence of a maternal relative of a missing person may facilitate identification of these human remains.

The FBI Laboratory developed the COmbined DNA Index System (CODIS) which combines forensic science and computer technology into an effective tool to provide investigative leads. CODIS enables federal, state, and local crime laboratories to exchange and compare DNA profiles electronically, thereby linking crimes to each other and to convicted offenders, or missing persons to family members (or believed personal effects). CODIS has implemented a missing persons database and mtDNA profile searching software known as CODIS^{MT}. The central function of the software is to facilitate searching of a mtDNA nucleotide sequence developed from an evidentiary sample against one or more reference databases. There are two files or indexes contained in the missing persons database. One index is the Relatives of Missing Persons Index (ROMPI) File which houses reference DNA profiles. The samples are donated voluntarily from related individuals of missing individuals. These DNA profiles are for missing person identification only and cannot be searched against any felon or unsolved case files. The other index is the Unidentified Human Remains Index (UHRZ) File which houses DNA profiles from blood or saliva samples from discovered children of unknown identity or from unidentified human remains. The DNA profiles in the UP File will be searched against profiles in the UPRI File.

VII. Admissibility

Although the scientific community has spent over ten years developing, validating, and laying the foundation for the forensic use of mtDNA analysis, there will be challenges to the admissibility of mtDNA sequencing. It is the responsibility of prosecutors to clearly and concisely present the material in a simple and understandable fashion. The goal is to present an explanation of the technology so that a court can easily make a determination that, when properly performed, mtDNA analysis generates results accepted as reliable within the scientific community.

Not all jurisdictions have made an admissibility determination of mtDNA analysis and, therefore, challenges on the use of this important forensic tool will continue. Failure of a prosecutor to carefully prepare and clearly present evidence might result in unfavorable or unsound rulings and thereby cause confusion within the legal community. Moreover, such an unfavorable ruling will only lend credence to unsupported and undocumented criticisms about the forensic use of mtDNA analysis. No matter which admissibility standard is applied (i.e., *Frye*, *Daubert*, FRE 702, etc.), similar presentations of evidence should produce identical outcomes of mtDNA admissibility.

When preparing for an admissibility hearing, first and foremost is the application of the "KISS" principal: keep it simple and to the point. Do not complicate the explanation of the science and statistical interpretation of mtDNA analysis by litigating issues on DNA analysis that have already been decided. In fact, most, if not all, of the science of mtDNA analysis has already been accepted as scientifically reliable in admissibility hearings that have been conducted on nuclear DNA analysis. For example, there is no reason to revisit the underlying science or validation of DNA extraction or PCR technology. In preparing for the hearing, request a preliminary ruling from the court that will limit the scope of the hearing so that the issues for the court's consideration will be clearly defined.

Generally, there are two main issues for the court's consideration during an admissibility hearing on mtDNA analysis. The first is the basic science related to mtDNA analysis. The second is the statistical interpretation applied to the results of the analysis when a match occurs. Both issues are well-established in the scientific community, within and without forensic science.

The principles and underlying theory of PCR are the same for all DNA typing technologies. PCR technology can be presented as the same as that used in the analysis of nuclear DNA. The only differences are that the primers are targeted to areas of the mtDNA chromosome and the number of cycles during PCR vary slightly from that of nuclear DNA analysis. Neither of these are germane to the issue of admissibility.

It is desirable to qualify an expert, when one is used, on areas related to mtDNA analysis methods that may include, but not be limited to, molecular biology, population genetics, and/or forensic applications of mtDNA analysis. In some cases, an expert that can qualify on forensic nuclear DNA analysis may be useful to establish the similarities between nuclear and mtDNA analyses.

Presentation by an expert can cover some or all of the following topics: 1) a basic explanation of the biology of the cell; 2) a basic explanation of DNA; 3) a basic explanation of the salient features of mtDNA; 4) a description of differences and similarities between nuclear DNA and mtDNA; 5) establishing that mtDNA is only inherited maternally and that all relatives who share the same maternal lineage typically possess identical mtDNA types; 6) a demonstration that the same principles apply in mtDNA profiling as in nuclear DNA profiling. The different steps: extraction, amplification, quantitation and sequencing are used throughout the molecular biology field. For mtDNA sequencing, the sequencing step determines the order of the bases along the mtDNA molecule. The differences in the sequence between or among individuals are called "Sequence Polymorphisms". Basically, sequence polymorphisms can be described using

an analogy to the information contained in a telephone number. The Court will readily appreciate that the seven digits of a telephone number are arranged in a particular order. If the digits are arranged in another sequence, the result is a different telephone number. In a similar manner, the As, Ts, Gs, and Cs are arranged along the DNA molecule in a particular sequence and differences in the sequence array can be used to differentiate between individuals.

MtDNA analysis has been generally accepted within the scientific community, and this can be demonstrated by publications, peer reviewed articles, scientific presentations and expert testimony. The identification of ancient remains (24, 26, 27, 39, 40, 53, 54, 55, 57, 58, 59, 60, 74) and documentation of human rights abuses (22, 37) are two areas in which mtDNA analysis has been utilized extensively. It has also been generally accepted as reliable in performing evolutionary research (73). MtDNA typing was used to identify the remains of Tsar Nicolas and the Romanov family, to identify skeletal remains in mass graves (20, 33), and to type the bones of soldiers from the Vietnam and Korean Wars (30). The Armed Forces DNA Identification Laboratory used mtDNA analysis to assist in determining the identity of the Vietnam soldier in the Tomb of the Unknown Soldier (17).

The mtDNA molecule is fairly robust, compared to nuclear DNA. It can survive varied and harsh environmental insults (77). For example, mtDNA has been successfully extracted and successfully sequenced from cooked meat products (72) and the fecal matter of crab lice (43).

There is a history and wealth of scientific acceptance and reliance upon population genetics/ statistical analyses. Published articles and expert testimony describe the population databases used for inferences of significance of a mtDNA sequence match (7, 25, 38, 41, 44, 46, 47, 48, 49, 50, 51, 52, 61). Interestingly, to date, there have not been any peer reviewed articles which have criticized or questioned the manner in which the data have been collected or interpreted.

Since 1996, mtDNA analysis has been held admissible in courtrooms throughout the United States utilizing both the *Frye* and *Daubert* admissibility standards. The first case was that of *State v. Ware* in Tennessee where the trial court's admission of mtDNA evidence was upheld on appeal. *State v. Ware*, No. 03C01-9705CR00164, 1999 WL233592 (Tenn. Crim. App. Apr. 20, 1999). The Tennessee Court of Criminal Appeals made note in its decision that, based upon the testimony of an expert from the FBI DNA Laboratory, mtDNA analysis met the *Frye* standard for admissibility. In addition, courts in South Carolina, Florida, Michigan, Maryland, Pennsylvania, Connecticut, Washington, Georgia and California have all held mtDNA evidence admissible. In *State v. Council*, 515 S.E. 2d 508, 516-18 (S.C. 1999), the appellate court upheld the trial courts' admission of mtDNA evidence under South Carolina's Rules of Evidence. Several states have applied the *Frye* standard and determined mtDNA analysis is admissible. *See State v. Bolin,* 90-11832 (Hillsborough County, Tampa, FL May 14, 1999); *People v. Holtzer*, 98-7603-FC (Grand Traverse County, Traverse City, MI, Jun 10, 1999); *State v. Williams*, K-94-1073, K-98-765 (Arundel County, Annapolis, MD, May 6, 1998); *Commonwealth v. Dillon*, 97-CR-1575 (Lackawanna County, Scranton, PA Jun 30, 1998); *Commonwealth v. Rorrer*, 98-0320 (Lehigh County, Allentown, PA, Oct 22, 1998), *aff'd on appeal*; *State v. Torres*, CR98102538 (Windham County, Willimantic, CT Jan 21, 1999) (mtDNA ruled admissible at hearing; pending trial); *State v. Smith*, 96-1-00957-1 (Clark County, Vancouver, WA, Jan 21, 1999); *State v. Poole*, 97-CR-1874 (Douglas County, Douglasville, GA, Apr 28, 1999); *People v. Torres*, 97NF3169 (Orange County, Santa Ana, CA, Sep 21, 1999); *Adams v. Mississippi*, 2001 WL 410800 (Miss.App., Apr 24, 2001). Moreover, in *State of Wisconsin v. Carl Saecker*, 196 Wis. 2d 646, 539 N.W. 2d 336 (Table) (Text in WestLaw), unpublished disposition, 1995 WL 507601 (Wis. App. Aug 8, 1995), mtDNA evidence was used in a post-conviction analysis, and the result used to support exoneration of the defendant, who had

been convicted of rape in a jury trial in 1989 and was serving a lengthy prison sentence.

In the New York case of *People v. Ko*, Indictment No. 2449/98 (New York County, May, 2000), the court ruled that mtDNA evidence is admissible at trial of the defendant for murder and other related charges. However, the court ruled that it would not permit testimony about a statistical analysis of the significance of the match in the case without testimony regarding the foundation of the approach to be utilized. This decision clearly is not a rejection on the statistical application of forensic mtDNA analysis. The court qualified its decision by stating that it would not prevent presentation of the statistical analysis at trial should a sufficient foundation be laid. In *People v. Klinger*, 185 Misc. 2d 574, 713 N.Y.S. 2d 823, 2000 N.Y. Slip Op. 20450 (N.Y. Co. Ct., Sept 5, 2000) and *People v. Kee*, Indictment No. 6425/99 (New York County, 2001), there was expert testimony presented on the validity of the population database and, for forensic purposes, the basic statistical approach. The evidence demonstrated that the statistical approach is a method that has survived the test of time and is applied throughout the mathematic, scientific and medical community. Basically, the statistics involve the number of times a particular mtDNA sequence is observed in a database(s). This counting method is a basic statement of fact that is the simplest statistical approach. The size of the database should then be taken into account when estimating mtDNA sequence frequencies using standard sampling theory. The formulas that are applied to determine the upper bounds of confidence intervals have been generally accepted as reliable. Two such formulas are utilized; one, when a sequence has been seen before in the database, and the other, when a sequence has never been seen before in a data base (61, 68). To date, there have been no dissenting published articles on the use of this statistical approach as it is applied to the interpretation of the significance of a mtDNA match.

In a hearing conducted pursuant to Federal Rule 702 to determine the admissibility of mtDNA typing, this same statistical analysis was admitted in *United States v. Turns*, CR-2-99-104, by the Hon. James L. Graham, United States District Court, Southern District of Ohio. On January 24, 2000, Judge Graham stated as follows:

> Now, the statistical approach used in this case is one that appears in the literature, it appears specifically in a comprehensive paper on the use of mitochondrial DNA analysis published by the office of the armed forces medical examiner, and it is described in that report at page 32 as being a very conservative statistical approach to determining the probability or likelihood that there could be a match in the general population. The data base of known mitochondrial DNA has grown to its present proportions over a period of time. It presently consists of 2,426 individuals. As the data base has grown, the rate of matches has not significantly changed. It was interesting to me to note that in some of the early nuclear DNA cases, the courts also referred to a statistical analysis which was based upon a data base of contributors, and in one of those cases, the data base was 225 randomly chosen FBI agents. That was the 6th Circuit case of United States versus *Bonds*, 12 F.3d 540, which approved nuclear DNA testing. So, again, the use of a data base of what we might consider somewhat limited proportions in comparison to the total population is not unusual, and this was exactly the same approach apparently used in the nuclear DNA cases.

United States v. Turns, CR-2-99-104, January 24, 2000.

The predictive effect of the statistical analysis is based upon a formula which is apparently recognized in the scientific community and used in a variety of scientific contexts, and it has been used specifically here in the analysis of mitochondrial DNA results. The court concluded that it is an accepted and reliable estimate of probability, and in this case, it led to results, interpreted results, which substantially increased the probability that the hair sample was the hair of the defendant in this case.

Most recently, in *People v. Cong Van Than*, Case No. 00CR2325 (Denver County, Colorado 2001), the trial court employed a modified *Daubert* standard and held admissible mtDNA analysis together with the statistical interpretation. In addition, in the case of *State v. Pappas,* 256 Conn. 854, _ A.2d _, 2001 WL789737 (Conn., July 24, 2001), a trial courts' decision was affirmed admitting mtDNA typing and the statistical interpretation after a *Daubert* hearing. After a careful review of the evidence presented regarding the statistical significance to be accorded a match, the court held that the testimony was statistically sound and that it was likely to be helpful to the jury in assessing the probative value of the mtDNA evidence.

Based upon the aforementioned court decisions, which are clearly supported by scientific publications, an overwhelming foundation has been set forth which establishes that the statistical analysis applied to determine the upper bounds of confidence intervals has been generally accepted as reliable within the mathematic, medical and scientific community.

Finally, challenges to mtDNA analysis often focus on the issues of heteroplasmy and contamination. The scientific community is well aware of heteroplasmy and does not find it to affect the reliability of mtDNA profiling (3, 4, 15, 23, 28, 56, 70, 75). Forensic scientists take appropriate steps to address the limited issue which it presents. It clearly does not affect the general acceptance of mtDNA profiling within the scientific community and, under certain circumstances, can be used to enhance or strengthen the weight of a match.

Contamination of exogenous DNA during handling of the evidence is a concern during the analysis of mtDNA because of the sensitivity of the assay (as it is for any PCR-based assay). Contamination is a day-to-day consideration that forensic laboratories, and all scientific laboratories, have to address when using PCR. There are standard accepted laboratory practices to minimize contamination and monitor contamination if it did occur during the analysis (8). These practices are not unique to forensic applications. The distinction to be made is that the content of a sample, or what has taken place prior to its arrival at the laboratory, cannot be controlled by the laboratory. The condition of the sample is what it is upon arrival.

There are no new issues for the use and admissibility of mtDNA analyses that have not been addressed with admissibility litigation of nuclear DNA. Precedents created by nuclear DNA admission provide guidance and ample support for mtDNA admissibility.

VIII. Conclusion

In conclusion, mtDNA sequencing provides another useful tool for characterizing biological evidence. The methodology is particularly useful for analyzing substantially degraded or environmentally-insulted samples, such as old bones, or samples with very minute quantities of DNA, such as hair shafts. The data are being used for identifying missing persons or human remains. Lastly, there is a substantial foundation to demonstrate scientific and legal admissibility of mtDNA typing.

IX. Acknowledgment

This is publication number 01-19 of the Laboratory Division of the Federal Bureau of Investigation. Names of commercial manufacturers are provided for identification only, and inclusion does not imply endorsement by the Federal Bureau of Investigation.

X. References

1. Allen, M., Engstrom, A.S., Myers, S., Handt, O., Saldeen, T., Von Haeseler, A., Pääbo, S., and Gyllensten, U. *Mitochondrial DNA sequencing of shed hairs and saliva on robbery caps: sensitivity and matching probabilities.* J. Forens. Sci. 1998; 43:453-464.

2. Anderson, S., Bankier, A.T., Barrell, B.G., de Bruijin, M.H.L., Coulson, A.R., Drouin. J., Eperson, I.C., Nierlich, D.P., Roe, B.A., Sanger, F., Schreier, P.H., Smith, A.J.H., Staden, R., and Young, I.G. *Sequence and organization of the*

human mitochondrial genomes. Nature. 1981; 290:457-465.

3. Bendall, K.E., Macaulay, V.A., and Sykes, B.C. *Variable levels of a heteroplasmic point mutation in individual hair roots.* Am. J. Hum. Genet. 1997; 61:1303-1308.

4. Bendall, K.E. and Sykes, B.C. *Length heteroplasmy in the first hypervariable segment of the human mtDNA control region.* Am. J. Hum. Genet. 1995; 57:248-256.

5. Bogenhagen, D. and Clayton, D.A. *The number of mitochondrial deoxyribonucleic acid genomes in mouse L and human HeLa cells.* J. Biol. Chem. 1974; 249:7791.

6. Boles, T.C., Snow, C.C., and Stover, E. *Forensic DNA typing on skeletal remains from mass graves: a pilot project in Guatemala.* J. Forens. Sci. 1995; 40:349-355.

7. Budowle, B., Wilson, M.R., DiZinno, J.A., Stauffer, C., Fasano, M.A,, Holland, M.M., and Monson, K.L. *Mitochondrial DNA regions HVI and HVII population data.* Forens. Sci.. Int. 1999; 103:23-35.

8. Budowle, Smith, J.A., Moretti, T.R., and DiZinno, J.A. *DNA Typing Protocols: Molecular Biology and Forensic Analysis.* BioTechniques Books, Eaton Publishing, Natick, MA. 2000.

9. Budowle, B. and Baechtel, F.S. *Modifications to improve the effectiveness of restriction fragment length polymorphism typing.* Appl. Theor. Electrophoresis. 1990; 1:181-187.

10. Budowle, B., Koons, B.W., and Errera, J.D. *Multiplex amplification and typing procedure for the loci d1s80 and amelogenin.* J. Forens. Sci. 1996; 41:660-663.

11. Budowle, B., Chakraborty, R., Giusti, A.M., Eisenberg, A.J., and Allen, R.C. *Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE.* Am. J. Hum. Genet. 1991; 48:137-144.

12. Budowle, B., Lindsey, J.A., DeCou, J.A., Koons, B.W., Giusti, A.M., and Comey, C.T. *Validation and population studies of the Loci LDLR, GYPA, HBGG, D7S8, and Gc (PM loci), and HLA-DQ∝ using a multiplex amplification and typing procedure.* J. Forens. Sci. 1995; 40:45-54.

13. Cann, R.L., Stoneking, M., and Wilson, A.C. *Mitochondrial DNA and human evolution.* Nature. 1987; 325:31-36.

14. Case, J.T. and Wallace. D.C. *Maternal inheritance of mitochondrial DNA polymorphisms in cultured human fibroblasts.* Somatic Cell Genetics. 1981; 7:103-108.

15. Comas, D., Pääbo, S., and Bertranpetit, J. *Heteroplasmy in the control region of human mitochondrial DNA.* Genome Research. 1995; 5:89-90.

16. Comey, C.T. and Budowle, B. *Validation studies on the analysis of the HLA-DQ alpha locus using the polymerase chain reaction.* J. Forens. Sci. 1991; 36:1633-1648.

17. Daoudi, Y., Morgan, M., Diefenbach, C., Ryan, J., Johnson, T., Conklin, G., Duncan, K., Smigielski, K., Huffine, E., Rankin, D., Mann, R., Holland, T., McElfresh, K., Canik, J., Ambrustmacher, V., and Holland, M. *Identification of the Vietnam tomb of the unknown soldier, the many roles of mitochondrial DNA.* In: Ninth International Symposium on Human Identification 1998, Promega Corporation, Madison, Wisconsin. 1998. http://www.promega.com/ussymp9proc/default.htm

18. Edwards, A., Civitello, A., Hammond, H.A., and Caskey, C.T. *DNA typing and genetic mapping with trimeric and tetrameric tandem repeats.* Am. J. Hum. Genet. 1991; 49:746-756.

19. Giles, R.E., Blanc, H., Cann, H.M., and Wallace, D.C. *Maternal inheritance of human mitochondrial DNA.* Proc. Natl. Acad. Sci. USA. 1980; 77:6715-6719.

20. Gill, P., Ivanov, P.L., Kimpton, C., Piercy, R., Benson, N., Tully, G., Evett, I., Hagelberg, E., and Sullivan, K. *Identification of the remains of the Romanov family by DNA analysis.* Nat. Genet. 1994; 6:130-135.

21. Gill, P., Jeffreys, A. J., and Werrett, D. J. *Forensic application of DNA fingerprints,* Nature. 1985; 318:577-579.

22. Ginther, C., Issel-Tarver, L., and King, M.C. *Identifying individuals by sequencing mitochondrial DNA from teeth.* Nature Genetics. 1992; 2:135-138.

23. Greenberg, B.D., Newbold, J.E., and Sugino, A. *Intraspecific nucleotide sequence variability surrounding the origin of replication in human mitochondrial DNA.* Gene. 1983; 21:33-49.

24. Handt, O., Richards, M., Trommsdorff, M., Kilger, C., Simanainen, J., Georgiev, O., Bauer, K., Stone, A., Hedges, R., Schaffner, W., et al. *Molecular genetic analyses of the Tyrolean Ice Man.* Science. 1994; 264:1775-1778.

25. Handt, O., Meyer, S., and von Haeseler, A. *Compilation of human mtDNA control region sequences.* Nuc. Acids Res. 1998; 26:126-129.

26. Handt, O., Krings, M., Ward, R.H., and Pääbo, S. *The retrieval of ancient human DNA sequences.* Am. J. Hum. Genet. 1996; 59:368-376.

27. Hanni, C., Laudet, V., Sakka, M., Begue, A., and Stehelin, D. *Amplification of mitochondrial DNA fragments from ancient human teeth and bones.* C. R. Acad. Sci. III. 1990; 310:365-370.

28. Hauswirth, W.W. and Clayton, D.A. *Length heterogeneity of a conserved displacement-loop sequence in human mitochondrial DNA.* Nuc. Acids Res. 1985; 13:8093-8104.

29. Higuchi, R.G., von Beroldingen, C.H., Sensabaugh, G.F., and Erlich, H.A. *DNA typing from single hairs.* Nature. 1988; 332: 543-546.

30. Holland, M.M, Fisher, D.L., Mitchell, L.G., Rodriguez, W.C., Canik, J.J., Merril, C.R., and Weedn, V.W. *Mitochondrial DNA sequence analysis of human skeletal remains: Identification of remains from the Vietnam War.* J. Forens. Sci. 1993; 38:542-553.

31. Holland, M.M. and Parsons, T.J. *Mitochondrial DNA sequence analysis -validation and use for forensic casework.* Forens. Sci. Rev. 1999; 11:21-50.

32. Hutchinson III, C.A., Newbold, J.E., Potter, S.S., and Edgell, M.H. *Maternal inheritance of mammalian mitochondrial DNA.* Nature. 1974; 251:536-538.

33. Ivanov, P.L., Wadhams, M.J., Roby, R.K., Holland, M.M., Weedn, V.W., and Parsons, T.J. *Mitochondrial DNA sequence heteroplasmy in the Grand Duke of Russia Georgij Romanov establishes the authenticity of the remains of Tsar Nicholas II.* Nature Genetics. 1996; 12:417-420.

34. Jeffreys, A.J., Wilson, V., and Thein, S.L. *Hypervariable minisatellite regions in human DNA.* Nature. 1985; 314: 67-73.

35. Jeffreys, A.J., Wilson, V., and Thein, S.L. *Individual-specific fingerprints of human DNA.* Nature. 1985; 316: 76-79.

36. Kasai, K., Nakamura, Y., and White, R. *Amplification of a variable number of tandem repeat (VNTR) locus (pMCT118) by the polymerase chain reaction (PCR) and its application to forensic science.* J. Forens. Sci. 1990; 35:1196-1200.

37. King, M.C. *An application of DNA sequencing to a human rights problem.* Mol. Genet. Med. 1991; 1:117-131.

38. Kogelnik, A.M., Lott, M.T., Brown, M.D., Navathe, S.B., and Wallace, D.C. *MITOMAP: a human mitochondrial genome database--1998 update.* Nuc. Acids Res. 1998; 26:112-115.

39. Krings, M., Stone, A., Schmitz, R.W., Kraintzki, H., Stoneking, M., and Pääbo, S. *Neanderthal DNA sequences and the origin of modern humans.* Cell. 1997; 90:19-30.

40. Kurosaki, K., Matsushita, T., and Ueda, S. *Individual DNA identification from ancient human remains.* Am. J. Hum. Genet. 1993; 53:638-643.

41. Lee, S.D., Shin, C.H., Kim, K.B., Lee, Y.S., and Lee, J.B. *Sequence variation of mitochondrial DNA control region in Koreans.* Forens. Sci. Int. 1997; 87:99-116.

42. Linch, C.A., Whiting, D.A., and Holland, M.M. *Human hair histogenesis for the*

mitochondrial DNA forensic scientist. J. Forens. Sci. 2001; 46:844-853.

43. Lord, W.D., DiZinno, J.A., Wilson, M.R., Budowle, B., Taplin, D., and Meinking, T.L. *Isolation, amplification, and sequencing of human mitochondrial DNA obtained from human crab louse, Pthirus pubis (L.), blood meals.* J. Forens. Sci. 1998; 43:1097-1100.

44. Lorenz, J.G. and Smith, D.G. *Distribution of sequence variation in the mtDNA control region of Native North Americans.* Hum. Biol. 1997; 69:749-776.

45. Lutz, S., Weisser, H.J., Heizmann, J., and Pollak, S. *mtDNA as a tool for identification of human remains.* Int. J. Leg. Med. 1996; 109:205-209.

46. Melton, T., Ginther, C., Sensabaugh, G., Soodyall, H., and Stoneking, M. *Extent of heterogeneity in mitochondrial DNA of sub-Saharan African populations.* J. Forens. Sci. 1997; 42:582-592.

47. Melton, T., Clifford, S., Kayser, M., Nasidze, I., Batzer, M., and Stoneking, M. *Diversity and heterogeneity in mitochondrial DNA of North American populations.* J Forens. Sci. 2001; 46:46-52.

48. Melton, T., Peterson, R., Redd, A.J., Saha, N., Sofro, A.S., Martinson, J., and Stoneking, M. *Polynesian genetic affinities with Southeast Asian populations as identified by mtDNA analysis.* Am. J. Hum. Genet. 1995; 57:403-414.

49. Melton, T. and Stoneking, M. *Extent of heterogeneity in mitochondrial DNA of ethnic Asian populations.* J. Forens. Sci. 1996; 41:591-602.

50. Melton, T., Clifford, S., Martinson, J., Batzer, M., and Stoneking, M. *Genetic evidence for the proto-Austronesian homeland in Asia: mtDNA and nuclear DNA variation in Taiwanese aboriginal tribes.* Am. J. Hum. Genet. 1998; 63:1807-1823.

51. Miller, K.W.P., Dawson, J.L., and Hagelberg, E. *A concordance of nucleotide substitutions in the first and second hypervariable segments of the human mtDNA control region.* Int. J. Leg. Med.. 1996; 109:107-113.

52. Miller, K.W.P. and Budowle, B. *A compendium of human mitochondrial DNA control region: development of an international standard forensic database.* Croat. Med. J. 2001; 42:315-324.

53. Oota, H., Kurosaki, K., Pookajorn, S., Ishida, T., and Ueda. S. *Genetic study of the Paleolithic and Neolithic Southeast Asians.* Hum. Biol. 2001; 73:225-231.

54. Oota, H., Saitou, N., Matsushita, T., and Ueda, S. *Molecular genetic analysis of remains of a 2,000-year-old human population in China-and its relevance for the origin of the modern Japanese population.* Am. J. Hum. Genet. 1999; 64:250-258.

55. Oota, H., Saitou, N., Matsushita, T., and Ueda, S. *A genetic study of 2,000-year-old human remains from Japan using mitochondrial DNA sequences.* Am. J. Phys. Anthropol.1995; 98:133-145.

56. Pääbo, S. *Mutational hot spots in the mitochondrial microcosm.* Amer. J. Hum. Genet. 1996; 59:493-496.

57. Pääbo, S., Gifford, J.A., and Wilson, A.C. *Mitochondrial DNA sequences from a 7000-year-old brain.* Nuc. Acids Res. 1988; 16:9775-9778.

58. Pääbo, S. *Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification.* Proc. Natl. Acad. Sci. USA. 1989; 86:6196-6200.

59. Pääbo, S., Higuchi, R., and Wilson, A.C. *Ancient DNA and the polymerase chain reaction. The emerging field of molecular archaeology.* J. Biol. Chem. 1989; 264:9707-9712.

60. Parr, R.L., Carlyle, S.W., and O'Rourke, D.H,. *Ancient DNA analysis of Fremont Amerindians of the Great Salt Lake Wetlands.* Am. J. Phys. Anthropol. 1996; 99:507-518.

61. Parson, W., Parsons, T.J., Scheithauer, R., and Holland, M.M. *Population data for 101 Austrian Caucasian mitochondrial DNA d-loop sequences: application of mtDNA sequence analysis to a forensic case.* Int. J. Leg. Med. 1998; 111:124-132.

62. Parsons, T.J., Muniec, D.M., Sullivan, K., Woodyatt, N., Alliston-Greiner, R., Wilson, M., Berry, D.L., Holland, K.A., Weedn, V.W., Gill, P., and Holland, M.M. *A high observed substitution rate in the human mitochondrial DNA control region.* Nature Genetics. 1997; 15:363-368.

63. Pfeiffer, H., Huhne, J., Ortmann, C., Waterkamp, K., and Brinkmann, B. *Mitochondrial DNA typing from human axillary, pubic and head hair shafts - success rates and sequence comparisons.* Int. J. Leg. Med. 1999; 112:287-290.

64. Saiki, R.K., Walsh, P.S., Levenson, C.H., and Erlich, H.A. *Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes.* Proc. Natl. Acad. Sci. USA. 1989; 86:6230-6234.

65. Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., and Arnheim, N. *Enzymatic amplification of beta-globin genomic sequences and restriction analysis for diagnosis of sickle cell anemia.* Science. 1985; 230:1350-1354.

66. Sanger, F., Nicklen, S., and Coulson, A.R. *DNA sequencing with chain-terminating inhibitors.* Proc. Natl. Acad. Sci. USA. 1977; 74:5463-5468.

67. Schneider, P.M., Seo, Y., and Rittner, C. *Forensic mtDNA hair analysis excludes a dog from having caused a traffic accident.* Int. J. Leg. Med. 1999; 112:315-316.

68. Snedecor, G.W. and Cochran, W.G. *Statistical Methods*, Iowa State University Press, Sixth Edition; 1974.

69. Southern, E.M. *Detection of specific sequences among DNA fragments separated by gel electrophoresis.* J. Mol. Biol. 1975; 98:503-517.

70. Stewart, J.E.B, Fisher, C.L., Aagaard, P.J., Wilson, M.R., Isenberg, A.R., Polanskey, D., Pokorak, E., DiZinno, J.A., and Budowle, B. *Length variation in HV2 of the human mitochondrial DNA control region.* J. Forens. Sci. 2001; 46:862-870.

71. Sullivan, K.M., Hopgood, R., and Gill, P. *Identification of human remains by amplification and automated sequencing of mitochondrial DNA.* Int. J. Leg. Med. 1992; 105:83-86.

72. Unseld, M., Beyermann, B., Brandt P., and Hiesel, R. *Identification of the species origin of highly processed meat products by mitochondrial DNA sequences.* PCR Methods Appl. 1995; 4:241-243.

73. Vigilant, L.M., Stoneking, M.., Harpending, H., Hawkes, K., and Wilson, A.C. *African populations and the evolution of human mitochondrial DNA.* Science. 1991; 253:1503-1507.

74. Wang, L., Oota, H., Saitou, N., Jin, F., Matsushita, T., and Ueda, S. *Genetic structure of a 2,500-year-old human population in China and its spatiotemporal changes.* 2000; Mol. Biol. Evol. 17:1396-1400.

75. Wilson, M.R., Polanskey, D., Replogle, J., DiZinno, J.A., and Budowle, B. *A family exhibiting heteroplasmy in the human mitochondrial DNA control region reveals both somatic mosaicism and pronounced segregation of mitotypes.* Nature Genetics. 1997; 100:167-171.

76. Wilson, M.R., Polanskey, D., Butler, J., DiZinno, J.A., Replogle, J., and Budowle, B. *Extraction, PCR amplification, and sequencing of mitochondrial DNA from human hair shafts.* Biotechniques. 1995; 18:662-669.

77. Wilson, M.R., DiZinno, J.A., Polanskey, D., Replogle, J., and Budowle, B. *Validation of mitochondrial DNA sequencing for forensic casework analysis.* Int. J. Leg. Med. 1995; 108:68-74.

78. Wyman, A.R. and White, R. *A highly polymorphic locus in human DNA.* Proc. Natl. Acad. Sci. USA. 1980; 77:6754-6758.❖

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Forensic Examination in Major Bombing Cases

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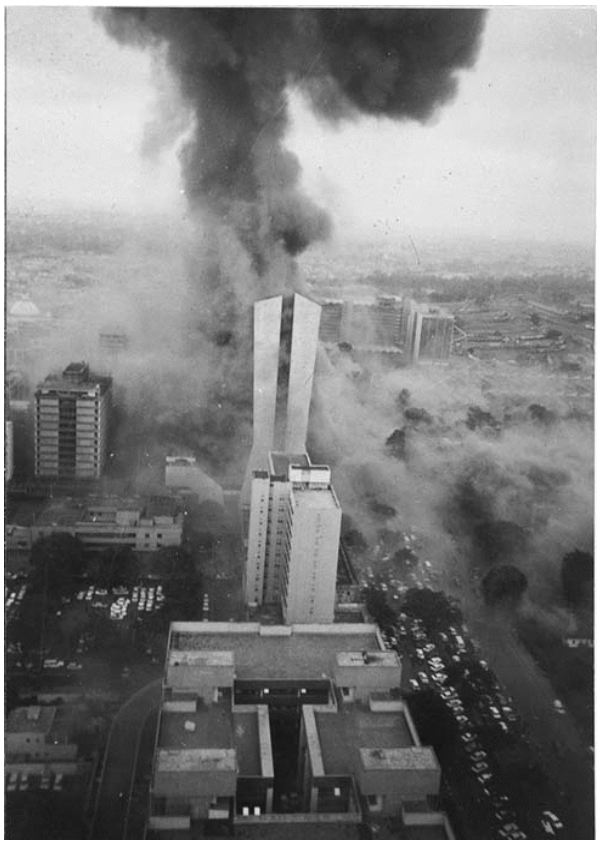
Although this article was in progress long before the events of September 11, 2001, the information that is included highlights the important work being done by law enforcement personnel following the recent terrorist attacks on the World Trade Center and the Pentagon and re-enforces the importance of cooperative partnerships with law enforcement in our antiterrorism efforts.

On November 13, 2000, the ethnic violence that plagued the Republic of Pomzania for the past one hundred seventy-five years spilled over onto United States soil. At ten-thirty on an overcast, fall morning, a rented van pulled up to the loading dock behind the Pomzanian consulate in downtown Indianapolis. About three minutes later the van erupted into a massive fireball. The force of the explosion caused the collapse of the rear of the consulate, killing three Pomzanian diplomats and two US citizens picking up visas at the consulate. The blast also shattered windows in nearby buildings, seriously injuring dozens of people in the area. The death toll would eventually reach nine.

Although the attack on the Pomzanian consulate is fictional, terrorist bombings have become an increasing problem around the world. The purpose of this article is to familiarize prosecutors with the process of collecting, examining and presenting evidence from a major bombing incident. Examples of recent major bombing incidents forensically examined by the FBI Laboratory include the World Trade Center (New York City - 2/26/93), Murrah Federal Building (Oklahoma City - 4/19/95), Saudi Arabian National Guard Building (Riyadh, Saudi Arabia - 11/13/95), Khobar Towers Building 131 (Dhahran, Saudi Arabia - 6/25/96), Nairobi Embassy (Nairobi, Kenya - 8/7/98), Dar es Salaam Embassy (Dar es Salaam, Tanzania - 8/7/98), and USS Cole (Aden, Yemen - 10/12/00).

All of these major bombing incidents involved the placement of a large quantity of explosives into a vehicle, then the detonation of these explosives inside, or next to, the intended target. However, a large explosive device does not need to be in a vehicle. In 1910, labor activists detonated dynamite in the Los Angeles Times building, killing twenty. More recently, terrorists in Russia have used long-delay timers to initiate caches of explosives that had been placed in rented storage rooms of apartment buildings. Nevertheless, the time and energy required to carry dozens of crates of explosives into a building greatly increases the risk of capture to a terrorist. Thus, a vehicle-borne bomb is a more likely choice, and will serve as the model for this case study.



Nairobi, Kenya, August 7, 1998

I. Responding to the Scene

The police officers, firefighters, and paramedics who responded to the ruins of the Pomzanian consulate faced a scene few had ever experienced. The one hundred year old building had collapsed in a heap of bricks, concrete, and glass. Listening devices detected several victims buried alive in the rubble. A frantic race began to remove debris and rescue the injured. As these rescue efforts continued, investigators looked for a reason for the catastrophe. Many believed that a natural gas leak had caused the explosion.

The first few hours after a large explosion are filled with chaos and confusion. Initially, a bomb scene may be treated as an accident site, rather than a crime scene. Rescue workers have a very limited amount of time to remove victims from the rubble. As a result, items relevant to the bomb vehicle may initially be overlooked or moved. Even after evidence is found to classify an incident as a criminal act, and a crime scene is established, the rescue of the injured takes precedence over the collection of physical evidence.

The Alfred P. Murrah Federal Building, Oklahoma City

The FBI Laboratory's Explosives Unit conducts training seminars for first responders to bomb scenes in which they stress recording the condition of the scene without interfering with rescue operations. The FBI Bomb Data Center has also published Investigators Bulletin 99-1, titled "Disaster Management", which outlines, for criminal investigators, the basic steps in processing a major bombing crime scene. Photographs taken of a bomb scene before rescue efforts have disturbed the area may be useful to the forensic explosives examiner in determining the location of the bomb vehicle at the moment of the explosion.

II. Determining Jurisdiction

Among the first responders to the Pomzanian consulate was an Indianapolis Police Department bomb technician. As this bomb technician approached the scene, he made a startling discovery. Lying in the middle of the street, almost two hundred yards from the consulate, was a twisted piece of metal over three feet in length. As the bomb technician studied this item, he realized that it was the rear axle from a vehicle. An image flashed into his mind of a photo he had seen, taken shortly after the bombing in Oklahoma City, that depicted an axle similar to one in the street in front of him. He felt certain that the explosion at the Pomzanian consulate had been caused by a vehicle bomb.

A meeting was hastily convened in the shattered lobby of a nearby office building. Present at the meeting were the heads of the Indianapolis Police and Fire Departments, along with the Special Agents in Charge (SAC) of the local Bureau of Alcohol, Tobacco and Firearms (BATF) office and the Federal Bureau of Investigation field division. Given the target and type of bomb, the FBI asserted its role as lead agency in the investigation of terrorist incidents. The FBI SAC called the United States Attorney and notified him of the incident. As this meeting concluded, the grim task of treating the wounded and retrieving the dead continued.

Determining which agency has investigative jurisdiction can be, at best, confusing and, at worst, fractious. Federal and state statutes overlap in bombing cases, especially where the victims have no direct nexus to federal interests. Our Pomzanian model is a typical mixed jurisdictional bag. While the assault on the diplomats is addressed by Title 18 U.S.C. § 112, assault on a foreign official, the U.S. citizens killed in the consulate and surrounding area, are covered by both state homicide statutes and federal bombing laws.

The solution to this problem is a joint investigative task force, working in close cooperation with the United States Attorney and local prosecuting authority. Under Presidential Decision Directive 39 (PDD 39), signed by President Clinton on June 21, 1995, the FBI serves as the lead investigative agency when a terrorist incident is suspected. PDD 39 also provides a framework for involvement by other federal investigative agencies, as well as local police forces. Many FBI field offices have Joint Terrorism Task Forces (JTTF) already in place, incorporating relevant agencies under one roof. These JTTFs help to ease the transition when a local disaster incident becomes a federal crime scene.

Axle from the bomb truck at Oklahoma City, located about 200 yards from the crater

The federal criminal code also provides a number of jurisdictional avenues in major bombing cases. The main explosives statutes are found in 18 U.S.C. §§ 841-848. For example, Section 844(d) prohibits the knowing transportation of explosives in interstate commerce with the intent to use them to damage or destroy a building. Another source of federal jurisdiction lies within the Antiterrorism Act of 1990, as amended, found at 18 U.S.C. §§ 2331-2339. The charges on which Timothy McVeigh was convicted were 18 U.S.C. §§ 2332a and 2(a) & (b), use of a weapon of mass destruction.

III. Collecting Evidence

As soon as the Indianapolis FBI SAC confirmed with the United States Attorney that federal jurisdiction applied to this case, she got on the phone with the Strategic Information Operations Center (SIOC) at FBI Headquarters. SIOC is the twenty-four-hour command post that occupies nearly half of the fifth floor at the J. Edgar Hoover building. After briefing the duty supervisor on her situation, and determining that the investigation would be named POMBOM, she made one request — send more resources. Within hours, forensic examiners, evidence collection specialists and bomb technicians were on their way to Indianapolis.

Before discussing how evidence is collected at the scene of a large explosion, it may be useful to briefly describe how explosives work. Explosives are broadly divided into two categories — low explosives and high explosives. Low explosives, such as the propellant used in bullets, can be ignited by heat, shock or friction. Something as simple as a burning match can start the reaction in low explosives that is known as deflagration. Deflagration, or rapid burning, produces gases which, if properly confined, can rupture their container, producing sharp fragments of metal or plastic, causing death or serious injury. A misconception by some people unfamiliar with low explosives is that plastic or metal pipes filled with low explosives, and sealed at the ends, are merely "big firecrackers."

High explosives pack an even greater punch. Compounds such as trinitrotoluene, or TNT, a common military explosive, require more than just a match to detonate them. Blasting caps, also called detonators, first patented by Alfred Nobel in 1867, contain just a few grams of very sensitive explosive, known as primary explosives. The shock wave produced when a blasting cap is initiated can start a chain reaction among molecules in high explosives, resulting in the detonation of that material.

The effects of detonation of high explosives are devastating. Within milliseconds, temperatures produced by the explosion can exceed two thousand degrees Centigrade. Even more significant is the blast pressure wave created during detonation. As the bonds that hold the molecules together are broken, energy is released in the form of a positive pressure wave. To visualize this process, think of dropping a rock into a smooth body of water. When the rock hits the surface of the water, it creates a wave that travels away from the point of impact. Similarly, the pressure wave created during the detonation of high explosives travels outward from the point of detonation. Unlike the gentle ripples on the surface of a pond, this detonation wave can move at more than fifteen thousand miles per hour, over twenty times the speed of sound. Very few structures can withstand the impact of this pressure wave at close range.

Another aspect of detonation that affects a crime scene is the negative pressure phase. As the pressure wave moves out from the point of detonation, it pushes the atmosphere, creating a vacuum behind it. At some point, air will rush back in to fill this vacuum. It is, therefore, possible to see walls at a crime scene that have collapsed back *toward* the seat of the explosion. This phenomenon is caused by the negative pressure wave striking objects that were weakened, but not destroyed, by the positive pressure wave.

The effect of detonating 50 lbs. of TNT in a motor home

Once we understand how explosives work, we can better organize the collection of evidence at a crime scene. Tasks at a major bomb scene, which can range from providing toilets for the crime scene workers to bagging evidence, can involve as many as two to three hundred people. The lessons learned from New York, Oklahoma City, Dhahran, Nairobi, Dar es Salaam and Aden have brought about a system of evidence collection that centers around the Evidence Response Team (ERT).

The FBI began forming ERTs in the 1980's in response to a need for a more consistent approach to the collection of evidence. Team members, who include Special Agents as well as support personnel, initially receive eighty hours of training on collecting evidence and documenting operations at a crime scene. Most ERT personnel go on to take additional classes, including the thirty-five hour Post Blast Investigators Seminar that is sponsored by the FBI Laboratory's Explosives Unit.

Since 1998, the FBI Laboratory has enhanced the ERT program with the creation of Rapid Deployment Teams (RDT) for major cases. The RDT system provides additional resources when a case overwhelms the capacity of the field office ERT. The RDTs have stockpiled enough crime scene processing equipment to support four simultaneous major crime scenes.

FBI Laboratory forensic examiners are another asset deployed to major bombing scenes. These examiners assist the ERT in identifying potentially significant items of evidence and advising the agent in command of the scene on any questions about forensics. Examiners from the Explosives Unit can also begin examining the evidence at the crime scene, providing information of potential lead value to the investigative teams. Laboratory examiners are also a key part of the RDT system. When an RDT deploys, a senior laboratory manager accompanies the examiners and coordinates follow-on laboratory resources.

IV. Examining Evidence

On November 28, fifteen days after the blast tore through the Pomzanian consulate, the Indianapolis ERT leader declared the crime scene completed. The evidence log recorded over four hundred items seized at the crime scene, ranging from soil samples taken from the bomb crater to a wheel hub found on a rooftop almost eight hundred yards from the consulate. The next day a convoy of vehicles departed Indianapolis, carrying the evidence from the crime scene to the FBI Laboratory for further examination.

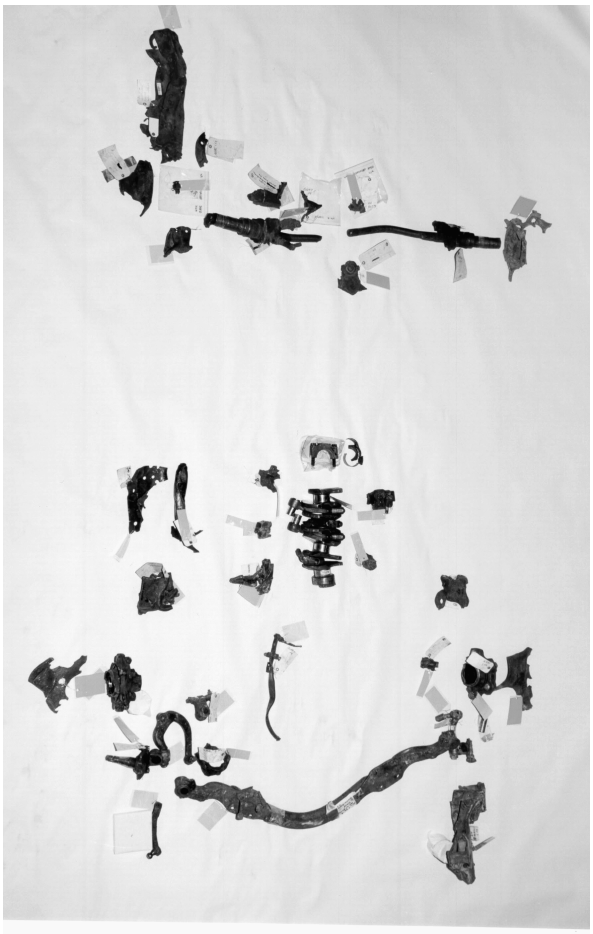
Examining evidence from a major bombing is like assembling a jig saw puzzle, except the pieces to this puzzle have been ripped apart by massive force and scorched by intense heat. The evidence starts its journey through the laboratory in the Explosives Unit. There, Explosives and Hazardous Devices Examiners, along with Physical Science Technicians, form an examination team to organize and catalog the evidence prior to distributing it among the relevant laboratory units.

The first order of business is preserving the chain of custody. Each discrete submission of evidence receives a laboratory number, whether it consists of four hundred fragments of a bomb vehicle collected at the crime scene or a single set of fingerprints taken from a suspect. The

Explosives Unit examination team then assigns Q or K numbers to each item, indicating whether the source of the specimen is Questioned or Known. Generally, in bombing cases, Q numbers are given to specimens recovered at the crime scene and K numbers are assigned to specimens seized from a known source, such as a suspect's residence. These Q and K numbers are used on the chain of custody forms to track specimens as they move from unit to unit within the laboratory. Photographs are also taken of the specimens before any examinations are conducted. These photographs record the condition of the specimen before tests are conducted that could alter its appearance.

With the evidence properly inventoried and labeled, the examination process begins. Just about every forensic discipline is brought to bear in a major bombing case. Chemists look for microscopic particles of explosives that remain on debris even after a detonation. DNA examiners process tiny fragments of skin, bone and teeth collected at a bomb scene to see if they can be matched to known standards from a suicide bomber. Even latent fingerprints have been known to survive an explosion. The majority of evidence collected at a major bombing scene, however, consists of pieces of the vehicle used to transport the explosives. The Explosives Unit has used automotive industry engineers to help identify these specimens. In the Nairobi embassy bombing case, an engineer from Toyota was able to positively match bomb truck fragments found at the crime scene to blueprints kept in the Toyota archives.

As each forensic examination is completed, the examiner prepares a Report of Examination with his findings. These reports are sent to the field office directing the investigation and the United States Attorney. After all examinations are finished, the specimens examined by the laboratory are shipped back to the field office for long term storage. Because major bombing cases typically involve several thousand specimens and dozens of laboratory numbers, good record keeping is essential. Each item of evidence could have several identifying numbers associated with it, ranging from the inventory number applied by the ERT at the crime scene, to a Q or K number designated by the laboratory, to an exhibit number given to it by the court clerk. Prosecutors should ensure early in the investigation that the field office case agent and laboratory CE develop a table or spreadsheet to correlate all these numbers.



Truck parts collected after Nairobi Embassy bombing

V. Identifying Suspects

On December 1, 2000, a woman with a heavy Pomzanian accent called the POMBOM reward hotline. She named Lazlo Harrglot as the mastermind behind the consulate bombing. A grand jury subpoena of Harrglot's telephone

records revealed several calls to fertilizer companies and van rental offices. Store employees picked Harrglot from photo spreads, identifying him as the purchaser of hundreds of pounds of fertilizer. Based on this information, a search warrant was obtained for Harrglot's residence in Indianapolis.

On December 5, 2000, a task force of FBI agents and Indianapolis police officers descended on Harrglot's home. The FBI ERT collected several items of interest, including blasting caps, batteries, spools of wire, half-empty fertilizer bags and various anti-Pomzanian literature. After consultation with the Assistant United States Attorney at the scene, Harrglot was taken into custody by the FBI and charged with one count of Title 26, Section 5861(d), possession of components from which a destructive device can be readily assembled. A joint JTTF/United States Attorney's Office press release was issued, announcing Harrglot's arrest but cautioning that it is too early to name him as a suspect in POMBOM.

It is human nature that, in any big case, everyone wants to be the person who solves the case. Forensic scientists have a legal and ethical obligation to refrain from being swept up in the emotions surrounding high profile cases. In this scenario, there would be tremendous pressure on the forensic examiners to link items found in Harrglot's house to the bomb at the consulate. The examiners must wait until they can conduct thorough examinations, in a proper laboratory environment, before reaching any conclusions. At most, a laboratory examiner would be at a search scene to offer advice on the types of items having forensic value. For example, forensic explosives chemists might accompany a search team to ensure that proper protocols are followed in collecting samples from the residence for analysis at the laboratory.

The situation at the Harrglot residence is similar to events that occurred during the search of Theodore Kaczynski's home. Kaczynski, who had been identified as a possible suspect in a series of sixteen bombings known as the UNABOM case, lived in a remote cabin in Montana. Investigation into his background revealed enough information to justify a search warrant, but not enough to give probable cause for an arrest. Upon entry into his cabin, the FBI Special Agent bomb technicians, including the author of this article, found a number of items of interest. Pipes, chemicals, wires, batteries and tools were all located in Kaczynski's cabin. FBI Laboratory examiners at the scene could not definitively link these items to previous UNABOM devices without more detailed examination. The bomb technicians could, however, describe these items as the basic building blocks for a bomb. Once BATF verified that Kaczynski had never paid the tax to register a destructive device, probable cause existed to place him in custody for possession of components from which a destructive device can be readily assembled.

The determination of what constitutes a destructive device or, in this case, components that could be readily assembled into a destructive device, has been the subject of some discussion among prosecutors. The definition of destructive device is found in both the Criminal Code at Title 18, Section 921, and the Internal Revenue Code at Title 26, Section 5845. The Internal Revenue Code requires anyone wishing to legally possess a destructive device to pay a tax and obtain a license from BATF.

In *Kikumura v. United States*, 978 F. Supp. 563, 585-86 (D. N.J. 1997), the court ruled that a witness who qualified as an expert in the field of bomb construction was competent to assist the trier of fact in determining if a defendant has made a destructive device. Examples of such expert witnesses include police bomb technicians and experienced military explosives ordinance disposal operators. FBI Explosives Unit examiners are often called as expert witnesses on the question of whether an item they examined constitutes a destructive device.

VI. Presenting Forensic Evidence in Court

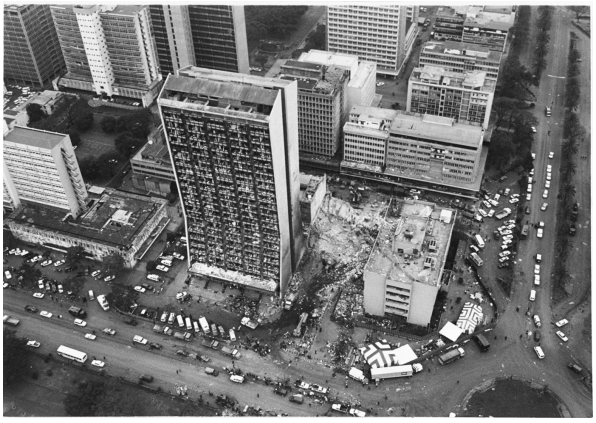
On January 3, 2001, a superceding indictment was issued charging Lazlo Harrglot with the

bombing of the Pomzanian consulate. Five months later he went on trial in the United States District Court for the District Of Indiana, facing charges ranging from assaulting foreign officials to the use of a weapon of mass destruction. The United States presented eighty-seven witnesses over a six week period. The defense case consisted of fifteen witnesses and concluded after just five days. Harrglot, who maintained that he was a political prisoner and not subject to the jurisdiction of the court, did not take the stand.

It is a daunting task for a prosecutor to have to choose which items, from the thousands of pieces of evidence collected during an investigation, best represent their case. Some specimens are obvious exhibits — a rental van receipt with the defendant's prints; a truck axle with the vehicle identification number, etc. On the other hand, parading four hundred pieces of twisted metal through the courtroom will numb the jury and exasperate the judge. One compromise is to introduce enough specimens to establish the identity of the vehicle and confirm its location at the crime scene. The explosives examiner's report will identify the specimens which exhibit the most significant explosive damage.

The sequence of the presentation of evidence is another factor to be considered. Typically, the first witnesses are the ERT personnel who seized the items at the crime scene. Next, if explosives residue was recovered, the forensic chemist would present his findings. Finally, the forensic explosives examiner would offer his opinion on the location of the bomb at the crime scene. If any parts of the fuzing system, such as a clock or batteries, have been identified, the forensic explosives examiner would present that information as well.

Visual aids also play a vital role in helping the jury understand complex forensic evidence. Clearly drawn diagrams and well-executed three-dimensional models, along with oversize photos of the crime scene, are frequently used to bring the enormity of a bomb scene down to manageable size. The FBI Laboratory's Investigative Support Section has a vast array of expertise and resources to create court room exhibits. The Coordinating Examiner can arrange for the preparation of these items.



United States Embassy, Nairobi, Kenya, August 7, 1998

VII. Conclusion

We can only hope that the Lazlo Harrglot's of this world find less destructive ways of promoting their political agendas. The headlines from around the world do not give much hope for such a change. Given the likelihood that violent acts of terrorism will continue to plague the free world, the aggressive investigation and prosecution of the responsible parties is imperative. We are blessed with a judicial system that protects the innocent and punishes the guilty. The forensic scientists at the FBI Laboratory are eager to assist the courts in the pursuit of these goals.❖

ABOUT THE AUTHOR

❑**Donald J. Sachtleben** is a Supervisory Special Agent assigned to the FBI Laboratory Explosives Unit since 1996 as an Explosives and Hazardous Devices Examiner. In his eighteen years with the FBI, Mr. Sachtleben has also served in the Pittsburgh and San Francisco field offices, conducting counter terrorism and bombing investigations. Mr. Sachtleben was certified as a bomb technician in 1990. He was an evidence team leader at the World Trade Center, Oklahoma

City federal building and Dhahran barracks bombings, as well as the coordinating forensic examiner for the Nairobi embassy bombing. He served as the Explosives Unit adviser in Aden, Yemen, after the bombing of the USS Cole. Mr. Sachtleben, authored an article in the November/December 1998 issue of Jane's *International Police Review* titled "Disaster Management."✠

Gunshot Residue Evidence

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I. Introduction

Gunshot residue (GSR) is the material that is generated by the primer composition of modern day ammunition. Today's center fire ammunition usually contains barium, antimony and lead compounds that can be deposited on a shooter's hands when the firearm is discharged. While these elements individually are all found in nature, it is very uncommon for them to be found together in anything other than gunshot residue.

GSR escaping from semi-auto. Note the fired bullet.

The precursor to existing gunshot residue testing was known as the dermal nitrate test. This was a hot paraffin casting of the hand that was tested with chemicals that gave a blue color reaction. This type of test was not specific, and a variety of contaminants interfered with the testing.

The next type of technology that was applied to gunshot residue testing was neutron activation

analysis. This required irradiating the swab samples taken from a suspect using a nuclear reactor and counting the decay time of barium and antimony to determine how much was present. Because of the cost and limited access to nuclear reactors, use of this method of testing was limited. In the early 1970's the Bureau of Alcohol, Tobacco, and Firearms developed the flameless atomic absorption (FAA) testing procedure for gunshot residue that is still in limited use today. The current preferred technology for GSR is Scanning Electron Microscopy (SEM) with x-ray analysis. This allows the examiner to see the particle morphology (spherical) and identify the elements present, non-destructively.

In 1994, the FBI stopped doing all gunshot residue testing for state and local authorities. The Bureau of Alcohol, Tobacco, and Firearms phased out its gunshot residue testing in 1999. Because of the overwhelming volume of work, and the reduction in cost of SEM and FAA technology, most state and local laboratories can now afford the necessary equipment.

II. GSR collection techniques

Most laboratories have commercially available gunshot residue kits for use by police officers in their jurisdiction. These kits should include gloves for the person taking the swabbings as well as all sampling materials and instructions to avoid contamination. In what are commonly called "Neutron Activation Analysis" kits for FAA testing, the shafts of the swabs should be plastic, not wood, and the test tubes should be plastic, not glass. Kits for SEM testing include SEM sampling stubs with adhesive affixed.

Gunshot residue consists of micro particulates that can be deposited on a suspect's hands or clothing. The transient nature of the particles makes detection/identification difficult. A suspect should not be fingerprinted prior to being swabbed for gunshot residue.

Gunshot residue evidence is recovered from suspects in three different ways. The first is to swab the individual's hands using cotton tip swabs and a diluted nitric acid solution (5% nitric acid). Several areas of the hand are swabbed with separate sets of swabs. Those separate swabs are packaged separately so they can be individually analyzed. At a minimum, the back of the hand and the palm area should be swabbed. In addition, if cartridge cases are available, a swabbing of the cartridge case is taken. Finally, a blank swab moistened only with nitric acid is taken.

The gases that escape when the firearm is discharged contain GSR and gunpowder residue. The hot gases disperse and condense on cooler surfaces such as hands, clothing, etc.

The second most common sampling technique is to use an adhesive material, and pat the hand surface with the adhesive to pick up the particulate matter.

A third method for sample collection is to rinse the suspects hands with a solvent and collect

the effluent. The first two techniques are generally considered effective and easiest to use. Gunshot residue collection should also be considered for the clothing or the face of the shooter. Positive results on the face area can substantiate witness statements that a rifle was used. To test clothing for gunshot residue, it is best to confiscate the suspect's clothing, and then submit the clothing in paper bags to the laboratory for testing, as opposed to swabbings by the officer. Typically, testing of clothing would only be done on the cuff area of shirts, or possibly the body of the shirt when a weapon is hugged close to the body during firing.

Most departments have guidelines that require sampling to be done within six hours; otherwise no testing for gunshot residue will be conducted.

III. Analyzing GSR, SEM, and FAA

The preferred technology for GSR is Scanning Electron Microscopy (SEM) with x-ray analysis. There are some laboratories that may still use flameless atomic absorption (FAA). Typically, laboratories test for barium and antimony. In addition, a number of laboratories will also test for lead, copper, or other heavy metals.

FAA will provide information about the elemental composition of the material that is being tested. SEM when coupled with energy dispersive x-ray (EDAX) provides particle morphology information, particle population and elemental composition about the gunshot residue. Due to the manner in which the particles are formed, gunshot residue particles are generally spherical, and the compounds of interest will all be located within one particle. With the scanning electron microscope, it is possible to visualize and test individual particles to make sure that all of the elements of interest are present. Having both elemental and morphology information increases the reliability for identifying gunshot residue.

Typically, SEM work takes up to several hours to run one sample, even if the operation is fully automated. There are a growing number of labs, public and private, that do GSR work, using SEM on a fee for service basis.

IV. Evidentiary value of GSR findings

The strongest conclusion that any forensic examiner can make, based upon gunshot residue testing results, is that the individual recently fired a firearm, handled a recently discharged firearm, or was adjacent to a gun when it was fired. It is not possible to conclusively say that the person fired the firearm, no matter what type of analytical testing is used. In certain scenarios, such as struggling for a weapon, gunshot residue may be present on both the victim and suspect's hands or clothing, although the location of the GSR may, in itself, have evidentiary value. As with most forensic testing, a negative finding by the laboratory does not indicate that the person definitively did not fire a weapon.

Most .22 caliber ammunition is rim fire, and does not contain barium or antimony salts in the primer composition. Newer center fire ammunition on the market also presents a challenge for gunshot residue testing because new primer compositions do not contain barium and antimony. GSR can be deposited downrange, so victims may be positive for GSR. Consequently, there may not be much evidentiary value in a positive GSR result from the victim, and they are not routinely tested for GSR.❖

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A Polygraph Primer for Prosecutors

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I. Introduction

Outside of clinical settings, the most widely used method of assessing a person's honesty or concealed knowledge is the polygraph. With the exception of intelligence testing, probably no area of assessment has received more scrutiny than that of polygraph testing. The polygraph has found utility in several venues including personnel security, certain industries (i.e., security officers, pharmaceutical manufacturing), and criminal investigations with witnesses, suspects, parolees, victims, and repeat offenders. In this primer we set out to cover polygraphy in the broadest sense from an applied and scientific perspective. We make no attempt to tackle the thorny legal issues in this brief article. The focus of this article will be the forensic application of polygraphy, and we hope it will provide an appreciation for the history of the polygraph, as well as enlighten the reader about the luminous future of the forensic detection of deception techniques. At the end of this article we list suggested readings that will add to the reader's understanding, in addition to Web addresses that could be useful.

If one is seeking controversy in the forensic arena, one needs look no further than polygraphy. The polygraph has been the source of controversy not just in the courts, but also in academic circles, on Capitol Hill, even on daytime television and movies (Ben Stiller in *Meet the Parents).* While the antagonists argue about whether the polygraph is too intrusive or if it usurps the role of the jury, the core disagreement is whether it is sufficiently accurate. If it is not as accurate as other accepted techniques, all other issues are moot. In forty years of pubic hearings and debate, polygraph critics and proponents have only succeeded in narrowing the range of accuracy to somewhere between chance and perfection. Certainly, both of the extreme camps are in error. It should be clear that a perfect lie detection technique does not exist. If it did, it would have already provided a solution for a host of social ills, perhaps even the elimination of crime itself. Conversely, if polygraph validity were as poor as the critics charge, it would not have survived nearly eighty years in the field, nor would the existing laboratory research have shown it to have a respectable accuracy. What we know is that the polygraph is fallible, but works well enough to be adopted by most police agencies, and dozens of federal agencies, for specific purposes. While we won't presume to know whether it is "sufficiently accurate," we will take up later what is known about its accuracy, and place it within the context of other commonly accepted diagnostic methods.

It should be pointed out early that, similar to many multidimensional assessment techniques, polygraphy is more complex than many recognize. It is not a single technique, but a family of techniques, each with specific strengths and shortcomings. Those considering using the polygraph should first weigh the relative benefit of a correct outcome versus the cost of an error. Also, it is important to clearly understand the goal. Is it to use the results for evidence; to help select police candidates; to catch spies; to facilitate treatment of convicted sex offenders; to locate a missing body; to validate a witness's information, or to help the prosecution or defense team decide upon a course of action? Each of these factors must be considered when deciding whether to use the polygraph, and what to do with the results.

II. Concise History

The first documented use of instrumentation to record physiological responses for the purpose of lie detection began at the end of the 19th century. It entailed the sealing of the examinee's hand in a container of water, and asking questions in an unsystematic fashion while observing the pressure changes in the container brought about by fluctuations in the examinee's blood pressure. Called the hydrosphygmograph, this device was reportedly used to resolve some important criminal investigations in Europe, though it fell into disuse by 1900, and never caught on in the United States.

Dr. William Marston devised another method in the early 1900s. Though it had nothing in common with the polygraph, it did involve, for the first time, the charting of physiological data for the purpose of lie detection. Marston's instrumentation consisted of a standard blood pressure cuff, which he used to take intermittent measurements of the examinee's blood pressure during questioning on relevant and irrelevant topics. He manually plotted these measurements of the blood pressure, creating a curve that was interpreted for assessing deception. Called the discontinuous blood pressure method, he taught it to the US Army, and it was used successfully in espionage investigations during World War I. In 1923 Marston attempted to have the results of his deception test entered into evidence in a murder trial in Washington, DC for defendant James Alphonso Frye. The well-known Frye Rule, (*United States v Frye,* 293 F. 1013, (D.C. Cir. 1923)), which was the first to consider deception tests, established the precedent for exclusion of lie detector results, an evidentiary standard since superseded by the 1993 Daubert decision (*Daubert v. Merrell Dow Pharmaceuticals*, *Inc.*, 509 U.S. 579 (1993)). Marston's discontinuous blood pressure method did not enjoy widespread field acceptance, with only three known practitioners, and there are no reports of its use after the 1930s.

The first true polygraphs used for lie detection were developed in the 1920s. They were bulky two-channel affairs that recorded blood pressure and respiration tracings on very large smoked drums. There were, at first, but a handful of examiners, and almost no research on polygraphy. Polygraphs continued to evolve over the decades as channels were added and deleted, components became progressively smaller, and smoked drums were replaced by pen and ink, now digitized displays. In contrast to its humble forebearer, today's laptop computer polygraphs record four or more channels of physiological data (two respiration, one cardiovascular, and one electrodermal) while the examinee undergoes a strict testing protocol. Complex algorithms, some federally funded, help polygraphers interpret the data. The polygraph profession has also grown. In the United States alone there are 4,000 or more practicing polygraph examiners in federal, state, local, or private employment, and possibly thousands more in the sixty-eight countries around the world known to have a polygraph presence. To supply instrumentation to these polygraphists, there are three domestic manufacturers of polygraphs, and at least four foreign models. Polygraphy has long since outgrown its obscure beginnings, now assisting investigative entities throughout the world.

III. The United States Federal Experience

Polygraphs were first pressed into federal service at the end of World War II, as part of investigations surrounding the Nuremberg Trials, and to enhance security at the new atomic processing facility at Oakridge, Tennessee. Government polygraph usage has grown slowly and steadily over the last six decades. There are currently twenty-three federal polygraph programs, and about 600 polygraph examiners in federal service. Though the role the polygraph plays varies across agencies, they fall roughly into three categories: criminal investigation, intelligence operations, and screening of applicants and employees. Some agencies have a single mission for their polygraph program, such as the Food and Drug Administration (FDA), where the polygraph is used to help solve product-tampering cases. The FBI uses the polygraph in all three roles, inasmuch as it screens new agents,

and has both counterintelligence and law enforcement responsibilities. The selection of role(s) depends on an agency's mission.

All federal polygraph examiners are highly trained. The government has centralized its polygraph training at Ft. Jackson, South Carolina, at the Department of Defense Polygraph Institute (DoDPI). Examiner candidates enroll from all parts of government. Federal standards require that a candidate must have a four-year college degree, federal investigative experience, and have undergone a screening polygraph examination, among other selection criteria, to be permitted to take the course at DoDPI. The course is 520 hours in length, taught at the Masters level, with those completing the program being eligible to receive credit toward a Masters degree through the American School of Professional Psychology. After the course, the new polygraphers undergo a closely monitored internship with their home agencies under the tutelage of senior polygraphers. There is also a continuing education requirement of eighty hours every two years to retain federal certification.

In addition to providing the training to all federal polygraph examiners, DoDPI also oversees quality control of federal polygraph programs. A cadre of DoDPI inspectors visit each program every two years, and review the program's records for evidence of compliance with federal standards. At the end of the week-long inspection, the inspectors issue a report that outlines the findings, and when there are deficiencies, programs are obligated to revise their practices and policies to meet the standards. This quality assurance process has ensured standardized practices across agencies, and improved the work product. It is the most thorough quality assurance process in existence for the profession.

DoDPI is also deeply invested in polygraph research, with large sums dedicated to future technologies that might eventually supplant the polygraph. Those replacement technologies will remain on the horizon for some time as the long process of basic research is completed. The polygraph is likely to continue as the mainstay of credibility assessment for the federal government for at least a decade, though science will likely bring about changes in the polygraph's components or methods of analysis.

IV. Polygraph Results

In criminal investigations, there are three possible polygraph decisions: Deception Indicated (DI), No Deception Indicated (NDI), or Inconclusive (or No Opinion in the federal government). A decision of DI and NDI require that the examinee be a suitable candidate for polygraphing, and that the physiological data are adequate, stable, and interpretable. Inconclusive outcomes result when at least one of these requirements is not satisfied. In multiple-issue screening examinations in the federal government, different terms are used. They are: Significant Physiological Responses (SR), No Significant Physiological Responses (NSR), and No Opinion.

V. How Polygraph Results Are Used

In both the criminal and screening domains, polygraph results are virtually never used alone. They are integrated into a decision process that incorporates other information and diagnostic methods. In the applicant screening environment, polygraph results and admissions are reported to hiring officials or adjudicators, who independently consider the polygraph information along with the results of the personal interview, resume reviews, background investigations, psychological testing, letters of recommendation, credit checks, or telephonic verification of the application information. Though the polygraph overwhelmingly provides more adjudicable information than all other methods, incorporating collateral sources helps maintain the integrity of the process, and prevent an overreliance on a single tool. In criminal polygraphy, results may be considered along with other forensic evidence, eyewitness accounts, victim statements, circumstantial evidence, the suspect's declaration, and the investigating officer's assessment, to determine whether to focus on a particular suspect. The polygraph in that setting is a means of optimizing limited investigative resources.

Sometimes those polygraph results will also help a prosecutor decide whether to drop or pursue a case. If the polygraph results were to be taken to the next level, and considered in a judicial proceeding, they are never used as the sole, or even the most important piece of evidence, but only to buttress other evidence.

VI. Polygraph Methods

The various polygraph examination protocols fall into three principal categories: Concealed Knowledge Techniques, Relevant-Irrelevant Techniques, and Comparison Question Techniques. Each operates differently and has unique applications. The following paragraphs describe how they came about, and how they work.

a. Concealed Knowledge Techniques

Concealed Knowledge Techniques are perhaps the oldest approach to lie detection. In the earliest part of the 20th century, decades before polygraphy emerged as a separate discipline, psychologists were experimenting with techniques for uncovering information that clients were concealing, and using these techniques for therapeutic purposes. The most popular were the word association test and reaction time tests. Word association tests enjoy some use by clinicians even today. These tests operated on the premise that critical stimuli are processed in a manner different from the processing of irrelevant stimuli, and this difference affects the outward behavior. This assumption is the underpinning of the Concealed Knowledge Techniques in polygraphy, where relative differences in the importance of stimuli elicit relative differences in the intensity of physiological reactions.

The family of Concealed Knowledge Techniques includes the Searching Peak of Tension Test (SPOT), the Known Solution Peak of Tension Test (KSPOT), and the Guilty Knowledge Technique (GKT). In each of these methods, a critical word or phrase is presented among others that are not related to the incident under investigation but would be equally plausible to a naive examinee. For example, if a murder victim wore a bright red shirt, and only the investigating officers and the murderer knew this detail, a test might be constructed like this: Do you know if the color of the victim's shirt was: white, blue, green, brown, red, black, gray? If the examinee physiologically responds greatest to the critical item (the color red in the previous example) it can be assumed that the examinee had knowledge that only someone close to the crime would have. In the case of the KSPOT and GKT, the examiner who constructs the list of stimuli is aware of which item is related to the crime. In the SPOT, and an alternate form of the GKT, the examiner does not know the true answer, and uses these techniques to determine what the examinee knows (i.e., location of other evidence).

The GKT is not often used in field polygraphy. Conditions under which the critical details of a crime have been sufficiently shielded from innocent examinees are fairly uncommon. Polygraphers have developed a preference for deception tests over knowledge tests like the GKT, since deception tests are not limited to only those cases where crime-relevant information has been adequately protected. Most polygraph examiners still employ deception tests even when the GKT could be used, an unfortunate bias, since even critics of polygraphy embrace GKT for evidentiary applications. Prosecutors considering using the polygraph as evidence should recommend the examiner employ the Guilty Knowledge Test if possible, as it will aid in obtaining scientific concurrence.

b. Relevant-Irrelevant Technique

The Relevant-Irrelevant (RI) technique evolved during the early years of polygraphy, in the 1920s. In that era, a fixed test question sequence, or even test protocol, had not yet evolved. Rather, polygraphers read relevant and irrelevant questions as they thought of them, with no particular order, and without reviewing the question wording with the examinee in advance. In his writings in the 1930s, Leonarde Keeler reported amazing success in detecting deception with the RI technique. He solved hundreds of crimes, enjoyed enviable press coverage, and he

even had his own radio show that featured some of his more famous cases. RI practitioners working in the criminal domain, however, began to discover errors in their decisions, and it soon became clear that the RI had a shortcoming: not just guilty examinees find the relevant questions more arousing than irrelevant questions. To complicate matters, there was no benchmark against which to compare reactions to relevant questions, so interpretation of the recordings relied primarily on the examiner's subjective assessments. In the 1950s and 1960s use of the specific issue RI in criminal cases began to wane as comparison question techniques emerged.

c. Comparison Question Techniques

In 1947, polygraph pioneer John Reid published a paper in which he suggested the inclusion of a probable-lie question in the question list so that a response to it could be compared to the response elicited by the relevant question. Called the "comparative response" question, this probable lie was tailored to the particular examinee so that he or she would likely lie to it. For example, if polygraphing an ex-convict for a murder, when he has a known history of burglary, a probable lie question might be "Since you got out of the penitentiary, have you committed any burglaries?" The goal was to create a condition such that an innocent examinee would be most concerned with the probable-lie question, and the guilty person the relevant question. Therefore, examinees could be categorized as deceptive or truthful to the relevant issues based on which type of test question they reacted to during testing. Reid's Comparison Question Technique (CQT) overcame the central problem associated with the RI format, because the comparison question created a benchmark for the interpretation of the physiological tracings.

There are several CQT formats in existence today. The two most common are the Zone Comparison Technique (ZCT), and the Modified General Question Technique (MGQT). There are several variants for both techniques, reflecting different schools of thought. Most of the current scientific research has focused on the ZCT.

Another development in polygraphy came about during the creation of the ZCT: numerical scoring. A 7-position scoring system, looking much like the Likert Scale, is now in general practice in the field. There are fixed scoring and decision rules, and current accuracy estimates are founded on decisions that result from the numerical scoring.

VII. Standards

Scores of standards have been promulgated within the polygraph profession and the federal polygraph community in the last ten years. The American Polygraph Association, the largest of the professional groups, has published standards of practice and ethical provisions, which are a condition of membership. Within the Federal Government there are technical standards for the agencies, and the biennial inspection ensures conformity. At the state level, roughly half of the states have enacted licensing laws, which regulate the profession. Outside both the government and the polygraph profession are the standards of the American Society for Testing and Materials (Committee E-52), which address training, ethical, technical, instrumentation, and research issues in polygraphy. Though compliance with the standards is voluntary, ASTM standards are regularly used as reference in civil and criminal actions, which acts indirectly to bring about better professional practices in the field. Direct enforcement of standards is incomplete across the polygraph profession. Federal standards only apply to federal programs and polygraphers, and a state licensing law affects only those practicing in that state. Therefore, many in the private sector operate where there are no uniform enforceable standards.

VIII. Accuracy

Determining polygraph validity has been an elusive goal for decades. It's not for a lack of data, as scores of studies have been published, but rather due to the unique problems associated with the scientific investigation of polygraphy. Polygraph research has taken two roads to validation: mock-crime laboratory studies, and real-crime field studies. Both seem to be

reasonable approaches, but each has a significant problem that limits what can be said about the true accuracy of polygraphy. In laboratory studies, experimenters instruct one group of their volunteers to act out scripted crimes, such as theft, murder, espionage, and sabotage, while the other group remains innocent of the acts. The volunteers are then polygraphed. Decision accuracy is based on the number of hits the polygraph has from both groups, which has averaged 85% - 95% across the studies. A major criticism of laboratory estimates of polygraph accuracy is that the volunteer examinees, usually paid a small fee for participation, do not have the same emotional experience as a criminal suspect facing a loss of reputation or freedom. Consequently, some scientists are skeptical of polygraph data produced in laboratory studies.

Field studies use polygraph data from actual criminal cases, thereby addressing the potential problem of the low emotional involvement of examinees in laboratory studies. Typically, experimenters collect cases in which it has been independently established that the examinee committed, or did not commit, the offense for which he or she was polygraphed. Those studies show an average accuracy range from about 75% to 95%. The major difficulty with field data is that only certain cases are resolved, and the polygraph is often a reason for that resolution. It has been argued that when the polygraph decision is correct with a guilty examinee, the examinee is confronted, and he will frequently confess. If the examinee was actually innocent, though the polygraph outcome says otherwise, the examinee will not confess, and the case may remain unresolved because the polygraph caused investigators to focus on the wrong individual. Since these types of cases remain unresolved, the cases can't be used for field validity studies, leaving a sample that might be different in some ways from all cases conducted in the field. While no one has yet proven a biasing of field samples, this remains one of the scientific criticisms that prevent acceptance of most field validation studies.

While any estimate of polygraph accuracy is still tentative, an independent researcher recently evaluated the available literature, and compared the best estimate of polygraph accuracy against the best estimate of accuracy of other diagnostic medical and psychological techniques. In this context, polygraphy's accuracy was about average. Below are data taken from that report. From Crewson, P.E., (2001) *A Comparative Analysis of Polygraph with Other Screening and Diagnostic Tools*, Report to the US Department of Defense Polygraph Institute (2001).

Table 1. Rank ordered "combined accuracy" of common medical and psychological diseases.

		Average Accuracy			
Condition	**Technique**	**Sensitivity**	**Specificity**	**Combined Accuracy**	**N of Studies**
Acute Appendicitis	CT	0.95	0.98	0.96	5
Brain Tumor	MRI	0.93	0.98	0.95	2
Acute Appendicitis	US	0.84	0.97	0.91	2
Breast Cancer	US	0.92	0.87	0.90	3
Deception	**Polygraph**	**0.92**	**0.83**	**0.88**	**37**
Breast Cancer	MRI	0.98	0.74	0.86	3
Multiple Sclerosis	MRI	0.73	0.93	0.83	2
Personality Disorders	DSM-IV	0.84	0.60	0.72	3
Depression	MMPI	0.68	0.65	0.67	25

Legend

CT = Computed tomography

MRI = Magnetic Resonance Imaging

US = Ultrasound

DSM-IV = Diagnostic and Statistical Manual, 4th edition

MMPI = Minnesota Multiphasic Personality Inventory

IX. Improvements

The forensic community and the justice system have a vital need for methodologies that can be used to verify statements made by an individual. Currently, the polygraph is virtually the only tool that can serve this function. However, despite its dominance in this area for much of the last century, the polygraph itself has not kept pace with scientific advancements. Improvements to conventional polygraphy are likely to emerge from three principal areas: automation, sensor technology, and signal analysis.

a. Automation

Inclusion of more automation in the polygraph examination process will provide a host of benefits, most of which are the direct consequence of the standardization offered by this approach. Reducing human interaction in the conduct of testing through automation minimizes potential bias, enhances validity and reliability, and reduces variability and human error in the performance of testing. It is important to point out that automation cannot ever replace a human in an assessment process. Rather, an intelligent combination of automation and skilled personnel can take polygraphy to its maximum capability.

b. Sensors

Polygraphy can also benefit from a fresh look at its sensors and source of information. Advancements in sensor technology are not well reflected in the configuration of current polygraphs, which have fallen behind other technologies in the physiological fields of study. Not only are more sensitive and comfortable sensors needed, there are many sensors as yet unexploited. Among the candidates for new measures are pupilometry, skin potential, thermography, pulse transit time, and impedance cardiography.

c. Signal Analysis

The government has underwritten developmental projects for automated analyses of polygraph data. The government-funded polygraph algorithm, and other commercially produced analytical tools, have performed well in cross validations. The principal use, and most successful application, of automated algorithms have been in single-issue polygraph testing. Accuracy of the algorithms with confirmed cases is about 90%, making them very useful in the field.

X. Future Improvements

The act of deceiving begins as a cognitive event in which the deceiver decides to communicate information to mislead someone. The cognitive process has neural underpinnings, but the neurological mechanisms involved in deception are not well understood. Central Nervous System or brain research is the next logical advance for the detection of deception. While most existing psychophysiological detection of deception methods, such as the polygraph, have looked for indicators well downstream from the cognitive event of deception, emerging technologies offer promise in identifying deception at its cortical source.

The most promising avenues for deception detection include the work with functional magnetic resonance imaging (fMRI), High Definition event-related potentials (HD-ERP) and Thermal Image Analysis. All three methods use patterns of activity to infer specific processes. Each uses advanced technology and automated analysis, and provides a unique perspective on mental processes. Spatial information, such as the location of activity within the brain, can be derived from images produced by the fMRI. If a brain location is uniquely implicated in the act of deception, the fMRI is a logical tool to determine whether that location has been activated. The presence or absence of activation in that specific region of the brain could be used as a deception indicator. However, this method requires very large and expensive equipment, restricting its deployability to the field. For this reason, scientists do not see fMRI as the first choice in new technology for detecting deception in the immediate future.

High Definition Evoked Response Potential (HD-ERP) uses scalp sensors to detect electrical signals from the brain. Already, the equipment is potentially portable, and could be developed into a turnkey system. It is one of the most promising new approaches on the horizon. ERPs, in contrast to fMRI, have already been used to detect guilty knowledge. Guilty knowledge tests, however, have very limited utility in the field. As a criterion for usefulness, a brain wave device must be capable of identifying deception with a high validity, a goal for current DoDPI-funded university research.

Thermal Image Analysis (TIA), the detection of patterns of heat from the skin, is a strong candidate technology. The application of TIA to the detection of deception may offer a non-invasive method for determining the veracity, or at least the emotional state, on an individual. With a parallel approach, Dr. Paul Ekman has shown that concealed emotions are revealed through “leakage” in facial expression, called microexpressions. The leakage is unconscious, and careful attention to it might be used to detect deception. Dr. Jeffrey Cohn has automated the analysis of facial expression. The marriage of TIA and Dr. Eckman’s theory may prove extremely beneficial to the development of this future sensor. Future research may also find this technology useful to augment other technical deception detection methods.

XI. Conclusion

If one conclusion can be drawn from this overview of the future of forensic detection of deception it is that it will cross several disciplines, and will be much larger than polygraphy as we have come to know it. Research in recent years is an acknowledgement of this trend. The instruments of the future will not likely resemble the instruments used today, and the examiners of the future will need to be trained to meet the sophistication of these instruments. The science of lie detection will only improve.

Suggested readings

Abrams, S., *A Polygraph Handbook for Attorneys* (1977).

Department of Defense Polygraph Institute. *Department of Defense Polygraph Program Annual Polygraph Report to Congress*. Ft. Jackson, South Carolina, (2000).

Eckman, P., *Telling Lies*, (1992).

Faigman, D.L., Kaye, D.H., Saks, M.J., and Sanders, J. (Eds) *Modern Scientific Evidence: The Law and Science of Expert Testimony*, (1997).

Kircher, J.C., Horowitz, S.W., & Raskin, D.C. *Meta-Analysis of Mock-Crime Laboratory Studies of Field Polygraph Techniques, Law and Human Behavior*, 12, (1988).

Kleiner, M. (Ed), *The Handbook of Polygraphy*, (2001).

Lykken, D.T. *A Tremor in the Blood, Uses and Abuses of the Lie Detector*, (1998).

Rogers, R.. (Ed), *Clinical Assessment of Malingering and Deception*, (1997).

Web Resources

American Polygraph Association Home Page: http://www.polygraph.org.
Lists available publications on polygraphy.

Department of Defense Polygraph Institute: http://www.dodpi.army.mil/
Official Web site of the Federal Government's central polygraph training, research, and quality assurance facility. Links to research abstracts.

National Academy of Sciences: http://www4.nas.edu/CP.NSF.
The NAS is reviewing the scientific evidence of polygraphy. Meeting agendas and minutes are posted at this site. A report is due in January 2002 National Polygraph Consultants: http://www.nationalpolygraphconsultants.com.
Commercial site with free searchable database of polygraph research citations. Polygraph Law Resource Page: http://truth.boisestate.edu/polygraph/polylaw.html.
Text of testimonies in polygraph-related cases, amicus briefs, judicial decisions.

Voice Stress: http://www.voicestress.org/
Lists summary of the available university-grade research on this polygraph alternative.❖

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Codes and Cyphers

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I. Introduction

The subject of secret codes and cyphers sets the mind racing with thoughts of cloak and dagger spies and dark conspiracies. One may conjure up the image of a secret agent in a dark room, painstakingly working by candlelight to convert a jumble of numbers into a coherent message. One may even think of a soldier wearing a headset eavesdropping on enemy radio signals. The mystery and intrigue of secret codes has always captured the imagination. Codes are most often associated with espionage and the military. Movies such as *Pearl Harbor* and *U-571*, along with recently published books about allied code breaking efforts during World War II have reinforced this association. While the use of codes and cyphers among espionage agencies and military organizations is well publicized, few may be aware of the prevalent use of codes and cyphers by another group: criminals.

II. History of Codes and Cyphers

Cryptology is the knowledge and study of all aspects of secret communications. Cryptography is the branch of cryptology concerned with the development and use of systems to protect communications. The two major categories of cryptographic systems are codes and cyphers. Cyphers involve the replacement of true letters or numbers with different characters. Codes involve substituting complete words, phrases or concepts with "code words." Encryption refers to the application of both codes and cyphers. The term code is used throughout this article to refer to both codes and cyphers.

The history of codes and cyphers in military and political applications is extensive. Since ancient times warriors have recognized that secure communications are essential to military success. Both the Greek and Roman empires developed cypher systems for their armies to communicate. Julius Caesar is credited with creating a cypher that bears his name to this day. The development of increasingly sophisticated cyphers and codes continued through the ages. The American Civil War saw both the Union and the Confederacy experimenting with various forms of encryption. Both armies also attempted to break the codes of their opponent.

The world wars during the 20th century saw some of the greatest achievements in both codemaking and codebreaking. During World War I British code breakers routinely broke German codes. The now infamous "Zimmerman Note" was a decrypted German diplomatic communication that discussed German-Mexican cooperation in the event the United States joined the Allies. The political fallout caused by the note was a key factor in America's entry into World War I. During World War II, the Allies' successfully solved the German's "unbreakable" enigma cypher. In the Pacific, American codebreaking efforts played a major role in the defeat of the Japanese Navy. In May 1942 the U.S. Navy intercepted and solved coded Japanese communications that revealed the time and location for the next enemy surprise attack. With this knowledge the commander of the Pacific fleet was able to position his forces to counter the strike. The resulting battle of Midway was a crushing defeat for the Japanese Navy and altered the course of the war.

Since World War II, advances in computer technology have radically changed cryptology. Gone are the days when armies relied on manually generated pen and paper codes and cyphers. Today cyphers are developed by computer

algorithms and are transmitted digitally. The development of the internet and public key encryption technology has eliminated the government's monopoly on encryption and brought advanced encryption capabilities down to the household level. But despite the cryptologic advancements of the information age, not all encryption users have chosen to abandon old-fashioned pen and paper codes and cyphers. Criminals remain steadfast users of manual encryption systems to conceal their illegal activities.

III. Criminal Codes and Cyphers

Criminals, like armies, are dependent on secrecy and have a long history of involvement with secret codes. The first widely publicized use of codes for criminal purposes was unveiled during the trial of Mary Stuart in 16th century England. Mary, and other defendants, were accused of hatching a plot to assassinate Queen Elizabeth. The key evidence at trial was a series of coded messages written by Mary to her conspirators. The Queen's secret police decoded the intercepted messages and thwarted the conspiracy. The decoded messages were such damning evidence that Queen Elizabeth extended no mercy to her sister. Mary Stuart was found guilty, along with the other conspirators, and beheaded in 1587.

Codes were involved in an assassination plot in American history as well. Following the murder of President Abraham Lincoln in 1865, the hotel room rented by the suspected assassin, John Wilkes Booth, was searched for evidence. Found among Booth's belongings was a matrix used for encoding messages. Union troops found an identical matrix in the Richmond office of a high ranking Confederate official. The matrixes were key evidence in the investigation to determine the Confederate government's role in President Lincoln's assassination.

The sophistication of codes used by criminals reached new heights during the prohibition era in the 1920's and 30's. Smuggling operations operating off the east and west coasts were importing a steady flow of illegal spirits using high speed boats that would rendezvous with large foreign vessels in international waters. These "rum runners" used nearly fifty separate and distinct code systems, including advanced code machines, to coordinate their smuggling operations. The United States Coast Guard and the Department of Commerce pooled their resources to intercept and decode the rum runners' messages. Between 1928 and 1930 approximately 12,000 messages were decoded. The decoded messages were instrumental to a number of federal criminal cases. In one case, decoded messages resulted in the federal indictment and prosecution of over one hundred people, including the ring leaders of the smuggling operations. The expert testimony provided by the cryptanalyst who led the codebreaking efforts was crucial to the success of the case.

Another well known prohibition era example is the case against Chicago mob boss Al Capone. Capone's eventual conviction on tax evasion charges was largely supported by evidence from coded ledgers. Federal agents successfully proved to a jury that coded entries in the ledgers represented payments for alcohol.

The most sensational criminal use of codes in recent history occurred in San Francisco, California during the summer of 1969. The self proclaimed "Zodiac" sent a three-part coded message to three bay area newspapers. In a separate letter to the editor the Zodiac claimed responsibility for several bay area homicides. The letter went on to say that the code contained the Zodiac's true identity. The police sought the assistance of the Federal Bureau of Investigation, Naval Intelligence, National Security Agency and even the Central Intelligence Agency. Having no success, the complete coded message was printed in an area newspaper on August 3, 1969. Within days a California history and economics high school teacher and his wife succeeded in what the government failed to do. In twenty hours of work the two amateur codebreakers solved the Zodiac's code. The decoded message revealed the killer's twisted motive for murdering but the true identity of the Zodiac was not revealed as stated in the letters, and the case remained unsolved.

IV. Criminal Codebreaking

Today, use of codes and cyphers by criminals remains a challenge to law enforcement. The ability to decode lawfully seized encrypted messages can give law enforcement a powerful tool in the investigation and prosecution of a case. The Federal Bureau of Investigation has recognized the value of criminal cryptanalysis and has dedicated an entire unit within the FBI laboratory to solve criminal codes and cyphers. The cryptanalysts of the Racketeering Records Analysis Unit (RRAU) of the FBI's Laboratory Division in Washington D.C. are at the forefront of a battle of wits between criminal codemakers, and FBI codebreakers. The RRAU is staffed with special agents and professional support personnel who are experts in various aspects of criminal cryptography. The RRAU examines coded documents in support of criminal investigations by federal, state and local law enforcement agencies.

The RRAU is organized into three subunits, each dedicated to a specific aspect of criminal cryptography. The cryptanalysis subunit examines all forms of manually encrypted letters or communications. The racketeering subunit examines coded records and documents seized from criminal organizations involved in illegal gambling, prostitution and loansharking. The drug records analysis subunit examines coded ledgers and documents from drug trafficking operations. Described below are the primary criminal activities encountered by the cryptanalysts of the RRAU.

V. Violent Criminals

Violent criminals use codes and cyphers for a variety of reasons. Some violent criminals, like the Zodiac Killer described earlier, use codes to taunt or intimidate. Encrypted threats directed at law enforcement officers or prosecutors are common. Threats are mostly encrypted using simple cyphers that are designed to be easily solved. In one partially encrypted threat letter to a state prosecutor the writer described the step by step procedures for solving the cypher.

Violent criminals also use codes and cyphers which are not meant to be read by anyone other than themselves. These personal notes or messages are typically encrypted with uniquely designed systems known only to the writer. Violent predators such as pedophiles and serial rapists have been known to use codes to record details about their intended victims. These "target lists" may include codes for the target's age, descriptive features, and other data pertinent to the writer. For example, the RRAU was tasked to examine the coded notes of a suspected serial rapist. The coded entries consisted of license plate numbers followed by a series of letters and numbers. Suspecting that the letters and numbers were codes to describe the vehicle's drivers, the RRAU cryptanalysts compared the coded entries with photographs of each vehicle's registered owner. The examination revealed that the codes represented female driver's hair color, length and other physical descriptors.

VI. Street and Prison Gangs

Gangs are, by far, the most prevalent users of criminal codes and cyphers. Gang members use a variety of different code and cypher systems with a wide range of sophistication. While the majority of gang codes and cyphers are relatively simple, some gangs go to great lengths to ensure secure communication. Gangs have used military field cyphers, ancient alphabets, obscure foreign languages, sign language and even made up languages to communicate. Some gangs use code systems which are virtually impossible to solve without access to the specific code keys.

In addition to codes and cyphers, prison gangs have developed various methods of concealing messages. Inmates have used lemon juice and urine as invisible ink. Messages have been found etched into the inside of envelopes and concealed within elaborate artwork. Gang members may write seemingly innocent letters with short messages embedded within the text. For example, every tenth word of a love letter may reveal a secret message. Concealment methods are limited only by the creativity and ingenuity of the

incarcerated gang members who have plenty of time on their hands.

VII. Racketeering Operations

Illegal businesses, like legitimate businesses, require record keeping. Gambling, prostitution and loansharking are illegal activities which require the maintenance of detailed records. These records are often coded to disguise the activity involved. For each bet placed with an illegal gambling operation, a record must be kept of the date of the wager, the wagering account name and account balance, the contest and line information, the amount of money wagered, and the results of the contest. Gambling records are typically coded for both secrecy and brevity. Decoding gambling records requires extensive knowledge of illegal gambling methods and terminology. Once decoded, the records have revealed the size and scope of the operations to include the number and roles of employees, number of bettors, and the type and volume of wagering activity.

Loansharking records are often coded to conceal the nature of the activity and the identity of borrowers. They may be disguised as records of legitimate business activities such as lay-away purchases. Decoded loansharking records have revealed the number of borrowers, the number and amount of loans, and interest rates.

Prostitution records are frequently coded to conceal the identities of prostitutes and the amount of money received. Records from operations that are disguised as escort services may contain coded items which reveal the true nature of the business. Decoded prostitution records have revealed the number of employees and their roles within the operation, fees, payments, and the volume of customers.

VIII. Drug Traffickers

Drug traffickers use a variety of different encryption systems in furtherance of their illegal activity. International smuggling operations have been observed using encrypted facsimile messages and telephone codewords to coordinate illegal drug shipments. Street drug dealers often communicate with their customers using codes entered into pagers and beepers.

Drug traffickers often go to great lengths to disguise records of drug transactions. Drug ledgers typically contain dates, products, weights, unit prices, purchase prices, and names. Drug names are often replaced with codewords to make the records look legitimate. For example, the ledger may contain records of a sale of cleaner #1 for $15.25. This could be a clandestine method of recording the sale of a kilogram of cocaine for $15,250. The coded ledgers used as evidence during the trial of Al Capone were coded in this manner. Money amounts may also be encyphered with symbols or letters representing numbers. Decoded drug records have revealed the number of individuals involved and the type and volume of drugs purchased and sold.

IX. Conclusion

Criminals have a long history of using codes and cyphers in furtherance of their criminal activity.

Law enforcement agencies and prosecutors should recognize the value of decoded messages in criminal cases, past and present. The FBI Laboratory Division's Racketeering Records Analysis Unit provides cryptanalysis support for federal, state and local criminal investigations. RRAU analysts are available for expert testimony, pretrial advice and assistance, and on-site examinations and consultations. For additional information, contact the RRAU at the following:

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X. Works Cited

Graysmith, Robert. *Zodiac*. New York: St. Martin's Press, 1986.

Wrixton, Fred B. *Codes, Cyphers, and Secret Languages*. New York: Workman Publishing, 1998.

Hornung, Rick. *Al Capone*. New York: Random House Value Publishing, 1998.

Fords Theater and the Lincoln Assassination. Alexandria, VA: Park's History Press, 2001.

Listening to the Rumrunners. Historical monograph. National Security Agency, Ft. George Meade, MD.❖

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