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### **Preservation and Collection of Biological Evidence**

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As the courts have placed greater emphasis on physical evidence during the past few decades, the initial stages of evidence examination have become increasingly important to the successful resolution of many criminal investigations. This emphasis on evidence collection and preservation is often manifested by many rigorous court challenges. This article reviews how the ability to introduce DNA test results in court is affected by methods used to recognize, document, collect, and preserve biological evidence.

Key words: base sequence; DNA; expert testimony; forensic medicine; genes; preservation, biological

During the past few decades, physical evidence has become increasingly important in criminal investigations. Courts often view eyewitness accounts as unreliable or biased. Physical evidence, such as DNA, fingerprints, and trace evidence may independently and objectively link a suspect/victim to a crime, disprove an alibi, or develop important investigative leads. Physical evidence may, also, prove invaluable for exonerating the innocent.

The initial stages of physical evidence examination can be pivotal to the successful resolution of criminal investigations. The methods employed in the recognition, collection, and preservation of physical evidence, such as DNA, have been rigorously scrutinized and challenged in court.

#### Sources of DNA

The forensic application of DNA typing methods over the past fifteen years constitutes a major advancement in the examination of biological evidence. With its remarkable sensitivity and power of discrimination, DNA analysis has become a key figure in the fields of forensic science, forensic medicine and anthropology, and paternity testing (1).

Many different types of physical evidence are commonly submitted to forensic science laboratories for examination. Initially, evidence that was suitable for DNA analysis was limited to biological substances that contain nucleated cells. This limitation has been overcome in the last 5 years with the implementation of mitochondrial DNA sequencing in the forensic arena. Common biological specimens from which DNA has been successfully isolated and typed are as follows: bones, blood and bloodstains, semen and seminal stains, tissues, organs, teeth, hairs, fingernails, saliva, urine, and other biological fluids.

The quantity of DNA that can be extracted from these common biological sources will vary (Table 1). Note that, in practice, crime scenes samples may contain considerably less usable DNA depending on environmental conditions. DNA has been isolated from other sources, such as gastric fluids and fecal stains. However, it can be difficult to generate a DNA profile from these sources in case samples due to significant degradation.

Several factors affect the ability to obtain a DNA profile. The first issue is sample quantity. The sensitivity of polymerase chain reaction- based (PCR) DNA typing methods is noteworthy, but still limited. The second concern is sample degradation. Prolonged exposure of even a large blood stain to the environment or to bacterial contamination can degrade the DNA and render it unsuitable for further analysis. The third consideration is sample purity. Most DNA typing methods are robust, and dirt, grease, some dyes in fabrics, and other substances can seriously compro-

| Type of sample          | Amount of DNA              |
|-------------------------|----------------------------|
| Liquid blood            | 20,000-40,000 ng/mL        |
| stain                   | 250-500 ng/cm <sup>2</sup> |
| Liquid semen            | 150,000-300,000 ng/mL      |
| Postcoital vaginal swab | 10-3,000 ng/swab           |
| Hair (with root)        | C C                        |
| Plucked                 | 1-750 ng/root              |
| Shed                    | 1-10 ng/root               |
| Liquid saliva           | 1,000-10,000 ng/mL         |
| Oral swab               | 100-1500 ng/swab           |
| Urine                   | 1-20 ng/mL                 |
| Bone                    | 3-10 ng/mg                 |
| Tissue                  | 50-500 ng/mg               |

affected by environmental factors.

mise the DNA typing process. Environmental insults will not change DNA allele "A" into allele "B", but they can adversely affect the ability of the scientist to obtain a complete DNA profile from the sample (2-4).

#### Evidence Transfer

DNA evidence can be used to make linkages or associations (e.g. person-person, person-other physical evidence, or person-crime scene). In general, biological evidence can be transferred by direct deposit or by secondary transfer.

#### Direct Deposit

Any biological evidence (blood, semen, body tissue, bone, hair, urine, and saliva) can be transferred to an individual's body/clothing, object, or crime scene by direct deposit. Once biological fluids are deposited, they adhere to the surface and become stains. Non-fluid biological evidence, such as tissue or hair, can also be transferred by direct contact.

#### Secondary Transfer

Blood, semen, tissue, hair, saliva, or urine can be transferred to a person, object, or location through an intermediary (person or an object). With secondary transfer, there is no direct contact between the original source (donor of the biological evidence) and the target surface. Secondary transfer may, but does not *necessarily*, establish a direct link between an individual and a crime. The impact of secondary transfer on the interpretation of DNA results has been debated (5,6). However, secondary transfer is clearly a more significant concern with the more sensitive DNA typing methods, such as mitochondrial DNA sequencing and low copy number PCR.

#### **Evidence Recognition**

The first step in a criminal investigation is determining which samples warrant further testing. This phase is crucial to the outcome of the investigation and very challenging, as crime scenes can be both complex and chaotic. Hence, an experienced investigator who systematically evaluates the scene is an invaluable resource. Recognition is the ability to identify probative evidence (at the scene or in the laboratory) scattered among potentially vast quantities of redundant, irrelevant, or unrelated items. For instance, collecting 20 bloodstains from the vicinity of a stabbing victim may not point to the perpetrator. The recognition process involves basic forensic principles, such as pattern recognition and analysis and physical properties observation. Naturally, if crucial evidence is not recognized, collected, and preserved, its value to the trier of fact will be lost.

#### **Documentation of DNA Evidence**

The location and condition of any biological evidence must be thoroughly documented before its collection. Careful evidence documentation at the crime scene, autopsy room, and forensic laboratory is essential. In any criminal or civil investigation, documentation has great bearing on whether the evidence can later be introduced in court. Evidence should not be processed or moved until its original condition and other relevant information have been recorded. Several different means of documentation are available. Generally, the use of more than one method is advised. The basic approach of evidence documentation and handling is outlined in Tables 2 and 3.

## Collection and Preservation of Biological Evidence

The ability to introduce DNA findings in court is also greatly impacted by evidence collection and preservation methods. Evidence integrity, both scientific and legal, begins with the first investigator at the crime scene. Detailed evidence collection protocols have been previously described (7-13). The specific collection method employed will depend on the state and condition of the biological evidence. In general, a significant quantity of material should be collected to ensure the recovery of sufficient DNA for testing purposes. However, it is important to limit collecting additional dirt, grease, fluids, and other material from the surrounding area, since many substances are known to adversely affect the DNA typing process. Each biological specimen should be packaged according to established forensic practices. Once the samples have been collected, they should be promptly delivered to the forensic laboratory. To minimize specimen deterioration, items should be stored in a cool, dry environment until they are submitted for testing.

Many famous investigations, such as O.J. Simpson and J.B. Ramsey, highlight the importance of effective crime scene processing (13,14). In the legal arena, unless the evidence is properly documented, collected, packaged, and preserved, it may not meet the legal and scientific requirements for admissibility into a court of law. If the DNA evidence is not properly documented before the collection, its origin can be questioned. If it is improperly collected or packaged, the possibility of contamination will be raised to discredit the DNA results. Given the prospect of legal challenges and the sensitivity of PCR methods, it is essential that strict contamination prevention measures be followed.

Legal concerns often diverge from empirical data. Even though PCR-based typing methods are sensitive, the contamination argument has been exaggerated in some cases (5,15). Moreover, it is important to note that, since all multi-locus DNA profiles (ie, >6 STR loci typed) are rare, contamination will predominantly lead to false exclusions or artificial mixtures rather than false inclusions. Consequently, albeit contamination could complicate result interpretation, it would typically not include the defendant.

#### **Challenges to DNA Admissibility**

Since their introduction into forensic science, DNA typing methods have been strenuously attacked in court (Table 4). Initially, the reliability of DNA typing procedures was questioned along with the statistical methods used to calculate DNA profile frequencies. In the last few years, legal challenges regarding the admissibility of DNA have shifted their focus away from the general reliability of the methods. Although most courts are comfortable with DNA testing in principle, some defense objections regarding DNA evidence continue to be effective. Successful challenges to the admissibility of DNA testing often address the initial collection, preservation, and subsequent handling of the biological evidence. The specter of evidence tampering may also be raised. Another common case-specific challenge concedes that DNA typing methods are reliable *in theory*. Here, the defense may suggest that critical mistakes were made in testing (sample switches, contamination, devia-

#### Table 2. Evidence documentation and collection

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|---|
| A. Evidence at a crime scene  |
| Photograph the evidence before it is touched, moved, or collected.  |
| Videotape the evidence and its relative position at the crime scene.  |
| Document the location and condition of the evidence.  |
| Note and sketch the spatial relationship of the evidence relative to other objects at the scene.  |
| Label, initial, and seal the evidence package.  |
| B. Evidence at the forensic laboratory  |
| Note the package, label, and seal condition of the item.  |
| Label the package with initials, unique case identifier, and date.  |
| Check the item number and compare it to the submission form to ensure that the correct item has been received. Also verify that the description of the item is accurate.  |
| Note, sketch, and/or photograph the contents of the package.  |
| Document the location and condition of biological evidence on the item prior to any sampling. Note when secondary cuttings of the evidence are taken; include the area where the cutting was collected. Package any sub-items separately. |
| When testing is conducted, record quantity of sample consumed, the test performed, and the results obtained. When handling the evidence, always wear clean, disposable gloves to minimize contamination.                                  |
| C. Evidence at the autopsy room   |
| Photograph the body and any additional evidence before cleaning the body.   |
| Note and sketch the evidence.   |
| Systematically collect each piece of evidence with clean tools.   |
| Separately package each item in a proper container.   |
| Label the container and note the quantity of sample collected. Do not add preservatives, such as formaldehyde to the specimen.  |
| Store the item appropriately.   |
| Carefully collect the clothing to avoid losing trace evidence and to avoid contamination with other biological samples.   |
| Release the evidence according to proper procedures.  |
|   |
| Table 3. Laboratory processing of DNA evidence  |
| A Laboratory receipt of avidance  |

#### A. Laboratory receipt of evidence

Physical evidence should be submitted to the laboratory with a transmittal letter, inventory sheet, and notation of the type of examination requested for each item according to standard laboratory protocols.

All identifying information on the physical evidence should be checked against the submission forms. Any discrepancies should be noted and corrected.

Each package should be properly packaged, sealed, and labeled. Any sign of improper packaging, sealing, or labeling should be noted.

Note any sign of sample leakage or contamination.

Any special requests/instructions regarding the DNA testing should be recorded on the submission form.

A receipt for evidence showing the date, time, submitting agency, submitter Is name, case number, item numbers, and the receiver Is name should be issued.

Physical evidence submitted for DNA analysis should be transmitted as soon as possible to the DNA unit and stored appropriately. B. Laboratory initial processing procedures

An evidence examination form should be used to record the preliminary processing of each item. It should contain the following data:

- a) Package description and actual contents;
- b) Label information, local case number;
- c) Description/condition of evidence;
- d) Laboratory case and item number;
- e) Date and initials of examiner.

Document the size, location, pattern, and condition of the stained area.

Weigh biological evidence, such as bone, teeth, nail, and tissue as necessary. Note the quantity used for DNA analysis. Record any preliminary test results and any trace evidence recovered.

Record information about each sample subjected to DNA analysis, as follows:

Case number, item number, and description; examiners[] initials;

- Reagent lot number; protocol followed; quantity of sample consumed.
- Testing results on each item should be entered on the appropriate worksheets.

Handle samples carefully to avoid mislabeling or cross contamination.

Whenever feasible, a portion of the sample should be preserved for possible future analysis. These specimens should be stored in a freezer. However, in many instances the item cannot be divided due to insufficient quantity. In this event, the sample should be processed according to standard forensic laboratory guidelines.

Any secondary cutting for DNA analysis should be placed in a separate container, package, or tube (labeled accordingly). Unused DNA should be properly labeled and stored in a freezer.

tions from laboratory protocols, misinterpretation of results, etc), which should invalidate the findings. With this strategy, typically the technical expertise of a particular laboratory or analyst is criticized.

Table 4. DNA admissibility challenges

- 1. Genetics issues
- 2. Procedural/technical issues
- 3. Results interpretation
- 4. Statistics
- 5. Contamination/other case-specific issues

#### Conclusion

The application of DNA technology in criminal investigations has grown rapidly in the past 15 years. DNA analysis has proven an extremely powerful weapon for both prosecution and defense. Throughout the world, DNA evidence has provided the critical linkages leading to numerous convictions. DNA's power as an exclusionary tool is equally noteworthy. However, DNA evidence that is not properly recognized, documented, collected, and preserved may ultimately be of no value to a criminal investigation. A greater appreciation of the importance of evidence collection and preservation is warranted or the forensic community may not be able to use this tool in the interest of justice.

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