

# MODERN METHODS OF COLLECTION AND PRESERVATION OF BIOLOGICAL EVIDENCE FOR HUMAN IDENTIFICATION BY DNA ANALYSIS

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## Abstract:

*The initial stages of physical evidence examination can be pivotal to the successful resolution of criminal investigations. In the present paper we intend to point and offer some guidelines for the methods employed in the recognition, collection and preservation of physical evidence used for DNA analysis.*

**Keywords:** DNA, physical evidence, human identification.

## 1. Introduction

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. [1,2]

Investigators and laboratory personnel should work together to determine the most probative pieces of evidence and to establish priorities. Given the sensitive nature of DNA evidence, officers should always contact their laboratory personnel or evidence collection technicians when collection questions arise.

Since the early 1990s, the advent of DNA profiling has vaulted biological crime scene evidence to a stature of importance that is only eclipsed by the fingerprint. In fact, the high sensitivity of DNA determinations has even changed the way police investigators define biological evidence. Today, the sensitivity of PCR means that 1 nanogram or less of DNA can yield sufficient information to individualize evidence. With this technology in-hand, the horizon of the criminal investigator extends beyond the traditional dried blood or semen stain.[4]

Table no.1 illustrates the power of DNA as a creator of physical evidence.

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| <b>Evidence</b>                  | <b>Possible Location of DNA on the Evidence</b> | <b>Source of DNA</b>                |
|----------------------------------|---|-------------------------------------|
| baseball bat or similar weapon   | Handle, end                                     | sweat, skin, blood, tissue          |
| hat, bandanna, or mask           | Inside  | sweat, hair, dandruff               |
| eyeglasses                       | Nose or ear pieces, lens                        | sweat, skin                         |
| facial tissue, cotton swab       | Surface area                                    | mucus, blood, sweat, semen, ear wax |
| dirty laundry                    | Surface area                                    | blood, sweat, semen                 |
| toothpick                        | Tips  | saliva                              |
| used cigarette                   | Cigarette butt                                  | saliva                              |
| stamp or envelope                | Licked area                                     | saliva                              |
| tape or ligature                 | Inside/outside surface                          | skin, sweat                         |
| bottle, can, or glass            | Sides, mouthpiece                               | saliva, sweat                       |
| used condom                      | Inside/outside surface                          | semen, vaginal or rectal cells      |
| blanket, pillow, sheet           | surface area                                    | sweat, hair, semen, urine, saliva   |
| “through and through” bullet     | outside surface                                 | blood, tissue                       |
| bite mark                        | person’s skin or clothing                       | saliva                              |
| finger nail, partial finger nail | Scrapings                                       | blood, sweat, tissue                |

**Table no. 1.** DNA as evidence

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 sensitivity of PCR DNA typing methods is noteworthy, but still limited. The second concern is sample degradation. Prolonged exposure of even a large bloodstain to the environment or to bacterial contamination can degrade the DNA and render it unsuitable for further analysis. The third consideration is sample purity and DNA content of different types of samples, as shown in table no.2.

| <b>TYPE OF SAMPLE</b>   | <b>AMOUNT OF DNA</b>       |
|-------------------------|----------------------------|
| liquid blood            | 20000-40000 ng/ml          |
| bloodstain              | 250-500 ng/cm <sup>2</sup> |
| liquid semen            | 150000-300000 ng/ml        |
| postcoital vaginal swab | 10-3000 ng/swab            |
| plucked hair with root  | 1-750 ng/root              |
| shed hair with root     | 1-10 ng/root               |
| liquid saliva           | 1000-10000 ng/ml           |
| oral swab               | 100-1500 ng/swab           |
| urine                   | 1-20 ng/ml                 |
| bone                    | 3-10 ng/mg                 |
| tissue                  | 50-500 ng/mg               |

**Table no.2.** DNA content of biological samples

## **2. Evidence collection**

All biological evidence is subject to deterioration. The careful collection and storage of this evidence will help ensure that this evidence is preserved so that useful information can be obtained from its analysis. Most DNA typing methods are robust, and dirt, grease, some dyes in fabrics, and other substances can seriously compromise 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 [7-10].

There are hundreds of varieties of physical evidence commonly submitted for examination to forensic science laboratories. Evidence that could be subjected to DNA analysis is generally limited to things that are biological in nature. The following is a list of biological materials from which DNA has been successfully isolated and analyzed:

- Blood and bloodstains
- Semen and seminal stains
- Tissues and cells
- Bones and organs
- Hairs with follicles
- Urine and saliva (with nucleated cells)

Other types of biological evidence, such as tears, perspiration, serum and other body fluids without nucleated cells are not amenable to DNA analysis.

Blood, semen, body tissue, bone, hair, urine and saliva can be transferred to an individual's body or clothing, or to an object or crime scene directly. Once liquid biological specimens have been deposited, they become stains and adhere to the surface or the substratum. Non-fluid biological evidence, such as tissue, bone or hair, can also be transferred by direct contact and deposit.

Blood, semen, body tissue, hair, saliva or urine could be transferred to a victim, suspect, witness, object or location through an intermediate medium. In a secondary transfer, there is no direct contact between the original source (donor of the DNA evidence) and the target surface. The transfer intermediary could be a person, object or a location. A secondary transfer does not necessarily furnish positive proof of a direct link of an individual with a specific crime.

Once the biological evidence is transferred through direct or secondary transfer, it will remain on the target surface either by absorption or by adherence. In general, liquid biological evidence will be absorbed, while solid state evidence will adhere. The method of collection depends largely on the state and condition of the biological evidence. The following are general guidelines for the collection of biological evidence for DNA analysis. 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.[3,5]

### ***Blood and Bloodstains***

If using the DNA profiling method of analysis, then blood and seminal fluid can be matched back to an individual with a high degree of probability. Currently, if using the PCR method of DNA analysis or conventional serological techniques then blood and some body fluids can be said to come from a certain population group to which the individual belongs.

### *Blood from a Person*

It is always necessary to collect reference samples from suspects and victims. In the great majority of cases, these samples consist of liquid blood.

Liquid blood from a person should be collected by qualified medical personnel. The crime laboratory should be informed if the subject had recently received a blood transfusion of any kind.

Two tubes of blood, about 5 mL each, should be collected in vacutainers with EDTA as anticoagulant. In the case of collecting reference samples from postmortem subjects, a blood sample should be obtained from non-body cavity areas such as heart or major internal blood vessels.

Each tube should be labeled with the date, time, subject's name, location, collector's name, case number and exhibit number.

Blood samples must be refrigerated, not frozen, and submitted to the laboratory as soon as possible.

### *Liquid Blood Specimens at Crime Scenes*

Liquid blood should be collected with a clean (preferably sterile) syringe or disposable pipette and transferred to a clean (preferably sterile) test tube. A blood clot can be transferred to a clean test tube with a clean spatula. A clean cotton cloth can be used to soak up liquid blood or a blood clot (avoiding areas containing only serum). Wet blood samples, if they are collected, must be preserved in a suitable anticoagulant and kept in a refrigerator. These specimens should be submitted to the laboratory as soon as possible.

Label the specimens with case number, item number, date, time, location, and evidence collector's name.

### *Wet Bloodstains*

Small objects bearing wet bloodstains should be allowed to air dry, then collected as is. An effort should be made to preserve the integrity of any bloodstain patterns during packaging and transportation.

Large objects that cannot be removed from a crime scene may have wet bloodstains on them. The wet blood should be transferred onto clean cotton cloth.

Bloodstained cotton cloth must be allowed to air dry before packaging in a paper container.

Each object and container must be properly labeled.

### *Dried Bloodstains on Removable Items*

Dried bloodstains on weapons, garments and other movable objects should be collected separately by collecting the entire item.

Each item should be placed in its own (paper) container, and these should be sealed and labeled properly.

### *Dried Bloodstains on Solid, Nonabsorbent Surfaces of Immovable Objects*

The bloodstain pattern should be documented and sketched to the extent necessary. The stain can be tape lifted or scraped off the object onto a clean piece of paper. The tape lifter or the paper with blood crust can then be placed into a "drugstore fold", and placed in an envelope which is sealed. Each item must be labeled properly.

If the bloodstain cannot be scraped off or the support object cannot be cut, then the bloodstain may be eluted onto a clean swatch, moistened with sterilized saline or water by rubbing

the cotton swab on the stained area. The swab is then allowed to dry and is placed in a paper fold packet. The packet is then placed in an envelope which is sealed, and properly labeled.

Always obtain a control by repeating the procedure on an adjacent but unstained area of the surface containing the bloodstain.

If the bloodstain is located on an object that can be cut, then a portion of the item containing the bloodstain can be removed by cutting with a clean, sharp instrument. Each cutting should be packaged separately and labeled accordingly. An unstained portion of the item should be collected and packaged as a control.

### ***Semen and Seminal Stains***

Document the semen evidence by notes, photography, videotape and sketching.

Use a clean syringe or disposable pipette to transfer liquid semen to a clean, sterile test tube. Label the tube with the case and item number, date, time, location, and name of the collector. Keep the specimen refrigerated and submit to the laboratory as soon as possible.

Alternatively, liquid semen can be transferred onto clean cotton cloth by absorption. The cloth is then air dried, packaged, sealed and labeled properly.

Seminal stains on panties, clothing, bedsheets, pillows and other movable objects should be collected as is. If an article has a wet stain on it, the stain must be allowed to air dry thoroughly prior to collection of the article. If the stain is on a large object that can be cut, then the stained area should be cut using a scalpel or scissors. If the stain is on immovable, nonabsorbent surfaces, then the stain should be scrapped using a clean scalpel onto clean paper, and fold the paper into a druggist fold container.

Each item should be packaged separately in a clean paper container. Each item's packaging must be properly sealed and labeled.

Packaged items should be refrigerated if possible, and submitted to the laboratory as soon as possible.

### ***Seminal Evidence from Sexual Assault Victims***

When dealing with sex crimes, the victim should be taken to the hospital immediately and the examination started as soon as possible. Photographs should be taken to document any injuries which the victim received. If necessary, oral, vaginal, and/or anal swabs should be taken from the victim and air dried for one hour in a moving air source as soon as possible. They should be collected as soon as possible because the body begins breaking down the various components in seminal fluid through drainage, enzyme activity, pH, etc. The swabs should be air dried under a fan for at least one hour. This can either be accomplished by the doctor at the hospital, or, upon collecting the kit from the doctor, the investigator should bring it immediately to a secure place and air dry it. The reason for this is that the moisture in the swabs allows microorganisms to grow which can destroy the evidentiary value of the swabs.

### ***Tissue, Organ or Bone***

Each item of evidence should be described in notes, and documented by photography, sketches or videotaping. This type of evidence item can be picked up with a clean pair of forceps. Each item should be placed in a clean container without any added fixatives.

Each container should be properly sealed and labeled, and stored in a freezer. Evidence should be submitted to the laboratory as soon as possible.

When collecting reference samples from postmortem subjects, if the body has decomposed, in addition to the blood sample, collect as many of the following specimens as possible: a portion of deep muscle tissue, certain organ tissue (e.g. heart or brain/not liver or kidney), 2-4 intact molar

teeth (if identification is an issue, ensure that mouth x-rays have been taken), and a sample of compact bone (e.g. femur). The specimens collected should be away from site of injury (i.e. if head injury, do not take sample of brain tissue). Immediately freeze specimens, do not place in any preservative (e.g. formalin).

Collection of *Urine, Saliva and Other Body Fluids* follows the same rules as blood and blood stains.

### ***Hair Evidence***

If a root sheath is attached, then DNA analysis using PCR technology can say that this hair came from a certain percentage of the population to which the suspect belongs. If there is no root sheath, then a microscopic analysis can say that the hair has the same characteristics as the suspect's hair and is similar to his or her hair.

Hair found at the scene should be placed in a paper packet and then placed in an envelope. If a microscopic examination is required, then 15-20 representative hairs from the suspect must be submitted to the lab for comparison. If DNA analysis is going to be used, then a whole blood sample from the suspect must be submitted to the lab in a "Vacutainer."

### **3. Transport and storage of evidence**

When transporting and storing evidence that may contain DNA, it is important to keep the evidence dry and at room temperature. Once the evidence has been secured in paper bags or envelopes, it should be sealed, labeled, and transported in a way that ensures proper identification of where it was found and proper chain of custody. Never place evidence that may contain DNA in plastic bags because plastic bags will retain damaging moisture. Direct sunlight and warmer conditions also may be harmful to DNA, so avoid keeping evidence in places that may get hot, such as a room or police car without air conditioning. For long-term storage issues, contact your local laboratory.

Refrigerate liquid blood samples (do not freeze). Air-dry all wet blood and other body fluid stains on evidence items (do not subject to heat). Until submission to the crime laboratory, freeze all stained items except for any metal or glass items (e.g. knives, bottles). Metal or glass items should be stored at room temperature and submitted to the laboratory as soon as possible.

Evidence from the suspect and victim must be handled and packaged separately.

### **4. Issues concerning contamination**

Because extremely small samples of DNA can be used as evidence, greater attention to contamination issues is necessary when identifying, collecting, and preserving DNA evidence. DNA evidence can be contaminated when DNA from another source gets mixed with DNA relevant to the case. This can happen when someone sneezes or coughs over the evidence or touches his/her mouth, nose, or other part of the face and then touches the area that may contain the DNA to be tested. Because PCR replicates or copies DNA in the evidence sample, the introduction of contaminants or other unintended DNA to an evidence sample can be problematic. With such minute samples of DNA being copied, extra care must be taken to prevent contamination. If a sample of DNA is submitted for testing, the PCR process will copy whatever DNA is present in the sample; it cannot distinguish between a suspect's DNA and DNA from another source.

Fortunately, an examination of DNA band patterns in the laboratory readily reveals the presence of contamination. For example with an STR, one will expect to see a two band pattern. If

one observes more than two bands, it becomes apparent that one could be dealing with a mixture of DNA from more than one source.

There are some relatively simple steps that crime scene investigators can take in order to minimize the possible occurrence of contamination of biological evidence:

- Wear gloves. Change them often.
- Use disposable instruments or clean them thoroughly before and after handling each sample.
- Avoid touching the area where you believe DNA may exist.
- Avoid talking, sneezing, and coughing over evidence.
- Avoid touching your face, nose, and mouth when collecting and packaging evidence.
- Air-dry evidence thoroughly before packaging.
- Put evidence into new paper bags or envelopes, not into plastic bags. Do not use staples.

Care should be taken to ensure that biological evidence is not contaminated during its collection:

- To avoid contamination, do not allow one evidence stain to come into contact with other biological samples.
- Do not talk or cough over biological evidence stains. Do not handle samples without using clean gloves.
- Each individual stain should be collected separately. Do not collect or package two separate stains together.
- Do not allow evidence samples to come into contact with any surface that contains residue from another biological sample (e.g. dirty tweezers, bloodstained glove, contaminated work surface).
- Use tweezers that have smooth, easy-to-clean working surfaces.
- Tools (e.g. tweezers, scissors) can be cleaned by thoroughly rinsing with a stream of distilled water and thoroughly drying with paper tissue. Repeat this process twice before using tool to manipulate another sample.

Package all biological evidence in paper bags or envelopes (do not use plastic). The packaging of biological evidence in plastic or airtight containers must always be avoided, because the accumulation of residual moisture could contribute to the growth of DNA-destroying bacteria and fungi.

- Allow stains to air dry as much as possible before placing in paper bag or envelope.
- Package the “unstained control” separately from the evidence stain.
- Package different evidence items in separate paper containers.
- Ensure that the paper container is large enough to allow air circulation around evidence item.
- Clean paper can be placed on (or in) a bloodstained garment and the garment folded so that the paper prevents contact between different stains. Ensure that while items are drying that the stain pattern(s) are not altered or the stain(s) cross-contaminated with other wet stain(s).
- Metal or glass evidence item (e.g. knife or broken, glass bottle), should be secured with wire to the bottom of a cardboard box so that it does not pierce the sides of a paper container. If not secured, blood on a knife blade can become easily dislodged and lost. Do not freeze metal or glass evidence items with blood or other body fluid stains. Submit these items to the laboratory as soon as possible.
- Tape seal, initial and date all paper bags or envelopes.

## 5. Conclusions

The dawn of a new age has arrived in law enforcement in the form of DNA research and testing. The specialists who work on the crime scene need to be aware of what they can do "in the field" to assure that proper evidence collection techniques are followed. The collection process will usually start with the collection of the most fragile or most easily lost evidence. Special consideration can also be given to any evidence or objects which need to be moved.

Biological evidence will attain its full forensic value only when an analyst can compare each of its DNA types to known DNA samples collected from victims and suspects. The ability to perform successful DNA analysis on biological evidence recovered from a crime scene depends very much on what kinds of specimens were collected and how they were preserved. Thus, the technique used to collect and document such evidence, the quantity and type of evidence that should be collected, the way the evidence should be handled and packaged, and how the evidence should be preserved, are some of the critical points for a forensic DNA testing program.

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