

# Forensic Biology (Serology & DNA)-Overview

**James Sebestyen, HQ Forensic Biology Manager, 404-270-8013**

The Forensic Biology discipline performs serological and DNA analyses of bodily fluids for the purpose of identification and individualization. The type of material typically examined includes, but is not limited to blood, semen, and saliva collected at crime scenes or from articles of physical evidence. These types of bodily fluids are frequently generated during the commission of violent crimes such as homicides, rapes, assaults, and hit and run fatalities. The ultimate goal is to identify what type of material is present and then, through the use of DNA analysis, link that material to a specific person.

A forensic biologist begins by evaluating the investigative information and available evidence to understand the nature of the case and the forensic question(s) to be answered. Initially, items of physical evidence are examined for blood, semen, or saliva. Further analysis is guided by the submitting agency's requests, case circumstances, sample size and condition and available technology.

Classification of biological evidence by conventional serology methods (ABO and polymorphic enzyme groupings) is no longer performed. DNA technology is used for individualization of biological evidence in forensic casework. Although biological evidence can be associated with an individual, it is not possible to determine the age of the sample.

Another service of Forensic Biology is the development of a Georgia DNA database of convicted felons and felony probationers as authorized by O.C.G.A. 35. This database allows for the comparison of DNA profiles from casework samples to those offenders in the Georgia file. Profiles are also periodically uploaded to a national database as well. This database utilizes a FBI program known as CODIS (Combined DNA Index System). Routine searches are performed of the Georgia database as well as at the national level. This is discussed in more detail under the "CODIS" section of this document.

Cases submitted with no known suspect will be tested and appropriate data entered into the CODIS system.

The following is an overview of the evidence submissions and services provided by Forensic Biology scientists in the examination and testing of evidence. The services include blood examination, saliva analysis, semen identification and DNA typing.

## Evidence Submissions

All forensic biology evidence must be submitted to the laboratory in a sealed and labeled condition or otherwise protected from loss of sample or contamination. Proper packaging includes paper or cardboard. Evidence should not be sealed in plastic, glass or metal as it can lead to the growth of mold and bacteria which can degrade biological evidence. Dried biological fluids are stable in an environmentally controlled, room temperature areas. Heat, humidity, and sunlight all have destructive effects on evidence. Only fresh tissue/bone, fetal samples or liquid blood samples should be stored short term at refrigerated temperatures.

## Collection and Submission of Known DNA Samples

A DNA comparison to DNA obtained from evidence can only be performed when DNA reference samples are submitted from known individuals. DNA reference samples (see below) may be in the form of liquid blood or buccal swabs (preferred). If other individuals are suspected to be involved in a crime, their known samples should be submitted when relevant. In sexual assault cases, if recent consensual sex (within 72 hours) is indicated, a known sample from the consenting partner is also necessary. Convicted offender samples collected in prison for purposes of the DNA database or CODIS cannot be used as a DNA reference sample.

### Buccal Swabs (preferred)

Buccal swabs are the preferred method of providing a DNA reference sample for DNA comparison. The buccal swabs are collected by rubbing swabs on the inner cheek of an individual's mouth. The swabs can be placed in a swab box or paper bag. Commercial kits may be purchased by private vendors if so desired. Instructions for collection of buccal swabs are contained in such kits and do not require special medical personnel. **NOTE: Do not collect a liquid saliva, urine or semen sample to be used a DNA reference sample. Gum, cigarette butts or drink containers are also not to be collected as known reference items.**

### Liquid Blood Samples

Blood (~5 milliliters) drawn for the purpose of being used as DNA reference sample should be in a purple stopper tube containing EDTA. The blood tube(s) should be adequately labeled prior to delivery to the DOFS laboratory with the full name of the person from whom the blood was collected, the date and time of collection, the name (or initials) of the person obtaining sample, and, when applicable, the name (or initials) of the investigator observing the collection. The tube(s) should be individually sealed and enclosed in packaging that is also sealed. Marking the outer package "Knowns for DNA" will ensure proper routing in the DOFS laboratory. **Note: Blood collected as part of a blood alcohol collection kit from a subject may not be used as a DNA reference sample.**

Blood samples should be forwarded to the laboratory at the earliest possible time following collection. When necessary, blood can be held refrigerated prior to transfer but must not be frozen. The blood tubes can be transported to the laboratory in a cooler but should not be submitted in a cooler due to the size. It is preferable that blood is delivered in person during working hours but can be mailed/shipped using priority delivery. The samples should be sent early in the week so that their arrival is not delayed by a weekend. Liquid blood samples must not be enclosed in the same package with other physical evidence.

Knowledge of a blood transfusion prior to collection of a known blood should be relayed to the analyst in written documentation accompanying the sample. If a person has received multiple blood transfusions, contact the assigned analyst prior to collection of a known sample. In this instance, it may be necessary to wait 90 to 120 days before collecting the known blood.

Bandages or other samples collected from a wound are not suitable for use as a known. When the individual is deceased, tissue or other appropriate samples should be collected at autopsy in the section below.

## Tissue and other Biological Samples from Autopsy

Samples collected at autopsy for use as knowns are dependent on the condition of the deceased. Blood or bloodstain cards are preferred but if unsuitable or unavailable, tissues (including pulled fingernails/toenails), bone with marrow or other biological samples may be submitted. Certain tissues are unsuitable, it is recommended that the Forensic Biology manager be contacted regarding the best-known sample source for analysis. These submissions must be adequately labeled with the sample type in addition to other identifying information. Samples preserved in formaldehyde (formalin) are unsuitable for DNA. Tissues should be refrigerated or frozen immediately to best preserve sample quality and transported to the DOFS laboratory on ice or priority shipped on dry ice.

## Crime Scene Evidence

When collecting crime scene evidence, it is preferable that the entire object be submitted to the laboratory with questioned stains intact. If removal or transport of an item is not possible, the stain(s) may be cut out or, when necessary, swabbed from the item. Small stains should be collected on a water-dampened cotton-tipped applicator that must be air dried prior to packaging. Due to the sensitive nature of DNA testing, care must be taken not to contaminate the sample by the individual collecting the sample. This includes wearing gloves, masks and refrain from sneezing or coughing on the sample.

Items from separate sources (victim and suspect clothing) or those suspected to have stains from different donors should always be packaged separately. All evidence items must be completely dried without use of heat or sunlight before packaging for delivery. Items with wet stains should be spread out on clear paper to dry because if they are folded wet, multiple stains can be created from one stain. Air-drying should be accomplished in a manner, which helps prevent cross-contamination of stains and loss of other evidence types (hair, fibers, or other trace evidence). Proper drying is particularly important to minimize the degradation of DNA.

## Sexual Assault Evidence Collection Kits

The GBI Sexual Assault Evidence Collection Kit is prepared commercially. These kits have been adapted to comply with recommended national guidelines and to meet the current protocols utilized at the GBI Crime Laboratory. The kit is sufficient to collect adequate samples from the victim's body to perform semen examination, saliva analysis or DNA analysis. The instruction sheet provided in each kit is explicit and complete for proper evidence collection. Inadequate collection and/or preservation of these samples can eliminate the potential for suspect identification. No tubes are provided to obtain a known blood sample from the victim during the medical examination. If blood samples are taken, they should not be placed inside the sexual assault kit itself and should be packaged separately as described above.

## Testing Times

The completion time for serological testing is highly variable and dependent on number and types of evidence items. The examination time for a single item is estimated below:

- Human blood – several hours to overnight
- Semen – several hours to overnight
- Spermatozoa (sperm) – several hours to overnight
- Saliva – hours

The longer time is usually needed when initial examinations are negative and require more extensive testing. Examination of multiple items and larger items will also increase these times significantly.

DNA analysis is a multiple step process with numerous quality control steps and validated protocols. The documentation and attention required by the procedures are essential to its success. DNA analysis typically can take up to 30 days. Contact Forensic Biology to discuss turnaround time for any priority cases.

## Blood Examination

When reasonable justification accompanies the service request, evidence will be examined to:

- Locate bloodstain(s)
- Identify blood by chemical testing
- Determine if of human origin (this analysis is not routinely performed. If blood is suspected to be of animal origin, provide that information upon submission.)

Blood examination on a victim's clothing or from under the body will not be performed when there is no question as to the blood's source. For the same reason, DNA testing on multiple bloodstains collected from a crime scene will not be performed unless there is justifiable reason to expect that more than one subject is bleeding. The fewest possible number of samples will be examined to answer the questions at hand. Weapons removed from a wound by medical or autopsy personnel will not be routinely examined for blood.

BlueStar® and Luminol are used by agencies to chemically detect the presence of latent blood. Application of these chemicals to the bloodstains negatively impacts the chemical examination performed by the GBI. Stains or evidence items processed by the agency using BlueStar® or Luminol will not be examined for blood however the items can still be evaluated for DNA analysis. The use of BlueStar® or Luminol should be noted upon submission.

## Saliva Analysis

When dried stains or questioned samples are submitted, the evidence items will be examined to:

- Locate stain(s) using alternate light source
- Identify amylase (a chemical component of saliva)
- Evaluate suitability of evidence for DNA analysis

When cigarette butts are submitted for saliva testing, the investigator should identify the brand(s) smoked by the victim, suspect, or other participants and confirm the last cleaning time of the location where collected.

## Semen Identification

When evidence is submitted in a sexual assault case with a suspect identified, the evidence will be examined as appropriate to:

- Locate the semen stain(s) visually or by alternate light source
- Perform chemical tests for seminal fluid
- Examine microscopically for spermatozoa (sperm)
- Test for other semen components when negative for sperm

When a sexual assault evidence kit from a male victim is submitted, if requested, the kit will be examined for semen. DNA analysis will be performed on cases that are positive for sperm. Sexual assault kits from a female victim with a male subject will be sent directly to DNA analysis as current technology allows for screening to be performed through the detection of male DNA.

Sexual assault evidence collection kits are typically examined first. Victim's clothing, bed linen and other intimate items can be evaluated for examination after the examination of sexual assault kit has been completed. Proper collection of victim's clothing remains important, however, because it can be needed for DNA analysis in some circumstances. Semen identification is not performed on suspect's clothing or bed linen.

# DNA Typing

DNA typing can be used as an investigative or trial tool to:

- Identify the donor(s) of biological evidence
- Exclude a falsely accused suspect
- Associate serial rapes or other series of similar crimes
- Determine victim identity when other identification is not available
- Determine parentage in criminal cases only (it is recommended to call the forensic biology manager to discuss this service prior to submission of samples.)

Over the years, the court challenges to the use of DNA technology have moved from the contention that the technology itself is somehow scientifically improper to attacks on the way the statistics are analyzed. The GBI Crime Lab has and will continue to interpret the statistics in a conservative and responsible manner.

## STR Typing

Forensic Biology utilizes commercially available STR (short tandem repeat) chemistries to develop DNA profiles for comparison to known individuals and for entry into the DNA database (CODIS). The current chemistry includes the 20 CODIS Core Loci as required by the FBI for NDIS participating laboratories. STR analysis is widely used for DNA identification. Generally, when a DNA comparison is performed and the evidence matches a known individual, a statistic called a frequency will be reported.

## Y-STR Typing

Sexual assault kits offer unique challenges with DNA evidence. When there is an overabundance of female DNA it can “mask”, or cover up, any male contributor. Y-STR typing utilizes short tandem repeats (STRs) found on the male specific Y chromosome. Females don’t have the Y chromosome and the female DNA is ignored with Y-STR typing.

Y-STR typing can also be used in non-sexual assault cases where mixtures are collected from evidence when there are high levels of DNA and low levels of male specific DNA. Other uses include but not limited to: exclude or include male suspects and identify paternal lineage of male perpetrators. Y-STR typing may be performed when specific criteria are met. For questions regarding this service contact the Forensic Biology Manager for further information.

## TrueAllele Casework Operations – Complex DNA mixtures

When complex STR mixtures are obtained from evidence and relevant DNA reference samples have been submitted, the DNA data can be analyzed using probabilistic genotyping software from Cybergenetics call TrueAllele®. Cybergenetics TrueAllele® technology, developed with MATLAB®, uses advanced statistical methods to extract identification information from DNA data. TrueAllele® is an automated computer system and software for interpreting DNA data. TrueAllele® can be used in some cases that have previously uninterpreted data if specific criteria are met. For questions regarding this service contact the Forensic Biology Manager for further information.

- [Cybergenetics TrueAllele](#)
- [MATLAB](#)

## Parentage Testing

Forensic parentage determination will be performed in criminal cases or in cases of unidentified remains. For criminal cases DNA reference samples from a “true trio” (mother, alleged father, child or product of conception) are required. For the purpose of identifying remains DNA reference samples from a first degree relative (i.e. biological father, mother or child, excluding siblings) must be submitted.

## Hair

DNA testing may be performed on hairs with visible roots. The GBI Trace section will examine the hairs and if suitable hair roots are present, cut the hair roots for DNA analysis. Hair roots contain a very small amount of DNA, therefore if any other probative forensic results are available, DNA analysis on hair will not be performed. For questions regarding this service, contact the Forensic Biology Manager.

## Fingernails

Investigative information must be available supporting the potential for biological material being present (victim or subject injured via scratching of nails, etc.). For requests to perform DNA analysis on fingernails contact the Forensic Biology Manager for further information.

## Contact DNA

Contact DNA is also known a touch DNA, low template DNA, Trace DNA or skin cell DNA. This category involves DNA transferred from a person to an item by that person having been in contact with that item. Although the DNA chemistries used today are more sensitive and require less sample, a certain amount of DNA is still required to obtain a reliable, reproducible result. Items that are repeatedly handled by a subject are preferred over items that have only been briefly touched. Worn items, such as shirts or hats, are considered contact DNA. Contact DNA analysis often yields complex mixtures which is not interpretable through traditional mixture deconvolution methods.

GBI will routinely perform contact DNA analysis on violent crimes (sexual assaults, homicides, armed robbery, etc.) and some burglaries (serial situation or extreme high dollar or value amount) without prior approval from management. In all other cases, Laboratory staff will evaluate the samples submitted and the case background to determine whether DNA analysis is the appropriate testing method or if alternative approaches such as latent prints or trace evidence may be more suitable.

## M-Vac Material Collection

The M-Vac uses a wet vacuum to collect biological material when traditional methods such as swabbing and cutting did not work. It can be utilized to process larger areas in one sample and to process difficult to swab areas. M-Vac testing may be performed when specific criteria are met. For questions regarding this service contact the forensic biology manager for further information.

- [M-Vac Material Collection](#)

## Genetic Genealogy

The GBI does not perform genetic genealogy analysis and the DNA data produced by the GBI is not the same as is used in genetic genealogy databases. Samples that yielded CODIS profiles from which no subject has been identified may be suitable for genetic genealogy analysis. If you have questions about what evidence items might be suitable, please contact the Forensic Biology Management team.

## **Combined DNA Index System (CODIS)**

CODIS is a FBI managed database of DNA profiles. These DNA profiles include casework and convicted offenders. Each state that contributes data to CODIS has its own definition of who is a convicted offender. The casework database is composed of “forensic unknowns”, which includes foreign DNA profiles from sexual assault cases and blood profiles from homicide, burglary, and assault cases. The FBI provides the software. Approved states may upload their profiles to the National Database to be searched against the database profiles from other states.

CODIS does not allow the users to upload profiles of victims, suspects, and elimination blood samples (e.g. DNA profiles of consensual partners in sexual assault investigations).

Any DNA recovered from evidence submitted to the GBI that did not originate from the victim or elimination person(s) and meets eligibility requirements is uploaded and searched in CODIS. If a potential match occurs (hit) between cases, the agency representatives involved will be notified and advised of any needed action on their part. If a hit is made with a convicted felon, the officer will be notified to submit a new known sample of the convicted felon for confirmation. All DNA profiles in CODIS are continuously searched against all new profiles added; therefore, there is no need to request a routine search in CODIS.