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Understanding DNA Testing and Reporting:
Processing Sexual Assault Kits

The information in this brief applies to cold case sexual assaults as well as current case sexual assaults. Mentions of sexual assault apply to both types of sexual assault cases.

Technological advances in DNA testing and leveraging the use of database searches in the Combined DNA Index System (CODIS) have, in part, driven testing of evidence from sexual assault kits (SAKs). If there is a police report or another form of documentation to show that a crime occurred, any foreign DNA obtained from evidence may be valuable to help identify a perpetrator and link with other crimes through a database search. Funding opportunities through the Department of Justice are available to test evidence from SAKs.

Significant efforts are being conducted to inventory and track both previously unsubmitted and currently submitted SAKs, but partially tested SAKs also may warrant reexamination. Older evidence still may be suitable for DNA testing, even if it was previously tested using biological fluid screening only or with early DNA tests, such as restriction fragment length polymorphism. The results of these examinations may even help determine whether there is viable evidence to proceed with additional testing using newer DNA technology. Even if evidence was partially or mostly consumed during prior testing, extracts and other byproducts of previous processes may be used.

CHALLENGE

Obtain CODIS-eligible DNA from serology-negative cases:

- DNA quantification and amplification kits are more sensitive for screening semen.
- Enzymes used in serological detection break down over time.
  - Not detectable if digital penetration or no ejaculation
  - Viable DNA still possible
- DNA extracts can provide viable DNA to test using
  - Expanded short tandem repeat (STR) loci kits
  - Y-STR loci kits

Out of 132 sexual abuse samples, 19 samples were positive for male DNA using Y-chromosome markers that previously screened negative using traditional serology techniques such as prostate specific antigen and microscopy techniques.[1]

(Stange et al., 2014)

Vulva and low vaginal swabs were recovered from a 19-year-old female 8 hours after an alleged sexual assault incident. No spermatozoa were detected. The samples were submitted for Y-STR testing and a full Y-STR profile was obtained.[2]

(McDonald et al., 2015)

STR Technology: Prominent DNA Test

DNA is an initialism for deoxyribonucleic acid. Found within the nucleus of the cells in our bodies, DNA is known as the biological blueprint of life. Nuclear DNA is passed down generation to generation, with half a person's DNA coming from each parent. Humans are very similar to other humans but, excluding identical twins, there are small differences in our DNA that can tell us apart. Forensic DNA testing focuses on the parts of the DNA that are different between humans.

Common forensic nuclear DNA tests look at short tandem repeats (STRs) in our DNA, where

- **short** refers to small segments of DNA,
- **tandem** refers to being right next to each other, and
- **repeats** means replication.

A forensic DNA test examines the number of times an STR repeats, known as an allele (see Example 1). Because half a person's DNA comes from each parent, each person will have two repeats (alleles) at each location tested (see Example 2). A DNA profile is created when many STRs (typically 15–24 locations) are examined. The DNA profile also indicates whether the profile is female (X,X) or male (X,Y).

Example 1. STR Profile at Three Marker Locations

STR Location	Allele 1	Allele 2
D8S1179	10	12
FGA	24	24
Amelogenin	X	Y

Note: In Example 1, D8S1179 is the name of an STR location examined. The results at this location are 10,12 (see Example 2). FGA is a second STR location examined, and the results are 24,24. Amelogenin is a sex-determining marker; the results X,Y indicate the DNA is from a male.

Example 2. STR Structure, D8S1179

TCTA-TCTA-TCTA-TCTA-TCTA-TCTA-TCTA-TCTA-TCTA-TCTA = 10 repeats
TCTA-TCTA-TCTA-TCTA-TCTA-TCTA-TCTA-TCTA-TCTA-TCTA-TCTA-TCTA = 12 repeats

STR technology is important in forensics. Because data can be obtained from a small amount of sample (i.e., pin drop), the regions are stable but also highly variable between humans, and the regions can be targeted simultaneously for efficiency. These features also make this technology popular for paternity testing, ancestry testing, and human identification in mass disasters and missing persons.

Expanded DNA Testing: Y-Chromosome and Mitochondrial DNA

Other types of DNA tests can be encountered in a forensic setting. Y-chromosome testing looks at STR regions on the male Y chromosome, which is passed down through the paternal lineage (i.e., from father to son). This testing can be useful when very little male DNA is detected in the presence of high amounts of female DNA. By focusing on the male DNA, forensic examiners can develop a Y-STR profile, essentially ignoring the impact of the female DNA. This makes Y-chromosome testing a viable option for detecting low levels of male DNA in SAKs.

Another type of DNA test uses mitochondrial DNA (mtDNA). Instead of looking at nuclear DNA, pieces of mtDNA—found within the cell but outside the nucleus—are sequenced to create an mtDNA profile. This type of DNA is passed down maternally (i.e., from mother to child) and is useful for testing hair shafts, where nuclear DNA is not present, or skeletal remains that often have compromised nuclear DNA. Due to inheritance patterns, Y-STR and mtDNA profiles cannot uniquely identify an individual, but these tests can provide very important information to help with an investigation.

Y-STR analysis has become an effective tool in aiding sexual assault cases. This technology can help resolve inconclusive STR results; determine the number of male contributors; and unmask a Y-STR profile that was concealed, during traditional STR testing, by the presence of female DNA.

DNA: The Laboratory Process

DNA is the same throughout all the cells in each person and commonly can be obtained from biological fluids (e.g., blood, saliva, and/or semen), hair roots, skin cells, tissue, and skeletal elements. Consequently, evidence submitted for possible DNA testing can vary greatly (see Example 3). A forensic examiner may attempt to identify a possible biological fluid or submit items directly to DNA testing. Using chemicals, the examiner will remove possible DNA from the submitted substrate (i.e., swab) and purify it. Next, the amount of human DNA retained from the item is determined; at this stage, the amount of male DNA present also can be established. Depending on the scenario, screening for male DNA is a vital process in some SAK workflows. If the amount of DNA recovered is below established detection limits, laboratories may choose to not proceed with further testing. However, by processing further, DNA is copied by targeting the STR regions that are different between humans. The STR regions are separated and, using computer software, the forensic examiner records the test result at each location and summarizes the results as well as any comparisons, conclusions, and statistics in a forensic case report.

Example 3. Items Commonly Submitted for DNA Testing

Bedding	Swabs from firearms
Bones	Fingernail scrapings
Bottles, straws, cups	Hair
Cigarette butts	Ligatures
Clothing: hats, shirts, pants, sneakers, underwear, gloves	SAKs: vaginal swabs, anal swabs, oral swabs
Swabs from surfaces: window, steering wheel, door	Swabs of possible stains: blood, saliva, semen
Condoms and wrappers	Weapon handles

DNA Reports and Conclusions

Forensic DNA reports have common, standardized elements that include report date, case identifier, description of the technology, DNA locations tested or chemistry utilized, description of the evidence examined, results, disposition of evidence, and the signature and title of the person authorizing the report. When applicable, conclusions and a quantitative or qualitative interpretation statement are included. If the case was screened for biological fluids (e.g., semen, blood, and/or saliva), a section or separate report will explain the screening, results and conclusions, and whether the item proceeded to—or is recommended for—DNA testing. For laboratories that screen SAKs with DNA, the lack or presence of DNA and decisions to further proceed with DNA testing also should be clearly communicated.

Depending on the amount of DNA and its quality, the result may have data at every location tested (i.e., a full profile) or data at some of the locations tested (i.e., a partial profile). If no DNA is detected—for example, no DNA is deposited on an item tested or the DNA is degraded due to varying conditions over time, temperature, and humidity—there will be no results. Profiles that contain data from more than one individual are referred to as DNA mixtures. Because one person may have up to two different alleles at each location, three or more alleles detected at a single location indicate multiple contributors. The totality of the profile is used to determine the results and make conclusions.

The DNA profile from an evidence item can be compared to known profiles obtained from the victim, suspect, or elimination samples. Results are commonly referred to as inconclusive, excluded, or included. When a result is *inconclusive*, there is typically not enough information, or the information is too complex to make a definitive conclusion; the DNA from that item is not reliable for making comparisons. An *exclusion* supports that a known profile cannot be contained within the profile generated from the evidence item, whereas an *inclusion* supports the known profile cannot be omitted from the DNA profile generated from the evidence item. Sometimes an inclusion also will be called a match when there is a single DNA profile from one individual. An inclusion or a match always should be supported with a quantitative statistical calculation that helps to explain the rarity of the inclusion (e.g., a random match probability or likelihood ratio).

Lack or presence of DNA should always be examined within the totality of all the evidence in an investigation. When there is an inclusion, a quantitative statistic represents the rarity of the DNA profile and cannot convey the chance the person committed or did not commit the crime. Thus, DNA cannot convey guilt or innocence. Currently, DNA evidence cannot determine the age of the DNA or the age of the donor, but research is being conducted in these areas.

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