Scientific Working Group on DNA Analysis Methods

Recommendations for the Efficient DNA Processing Of Sexual Assault Evidence Kits

The Scientific Working Group on DNA Analysis Methods, better known by its acronym of SWGDAM, is a group of scientists representing Federal, State, and Local forensic DNA laboratories in the United States and Canada. During meetings, which are held twice a year, Committees discuss topics of interest to the forensic DNA community and often develop documents to provide direction and guidance for the community. In some instances, an Ad Hoc Working Group may be empaneled to address a particular topic outside of the routine SWGDAM January/July meeting schedule.

Background

The Sexual Assault Forensic Evidence Reporting Act (SAFER Act) authorizes the Director of the National Instiute of Justice (NIJ) to develop "protocols and practice…for the accurate, timely, and effective collection and processing of DNA evidence, including protocols specific to sexual assault cases, which shall address appropriate steps in the investigation of cases that might involve DNA evidence." In response to this Act, NIJ created a working group of representatives of victims, victim advocates, sexual assault nurse examiners, law enforcement personnel, forensic laboratory personnel, prosecutors, and the judiciary to recommend best practices for cases involving DNA evidence. To assist NIJ, the SWGDAM Chair empaneled a Sexual Assault Forensic Evidence Reporting (SAFER) Working Group in February 2014 to provide recommendations specific to the laboratory's efficient DNA processing of sexual assault evidence kits. The SWGDAM SAFER Working Group met

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over a two and half year period and provided NIJ with recommendations for inclusion in the "National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach." The SWGDAM SAFER Working Group drafted this document to elaborate on those recommendations and provide additional detail on the laboratory specific topics related to the efficient processing of sexual assault evidence kits (SAKs). This document was provided to the SWGDAM body and the public for comment during the spring/summer 2016 and the SWGDAM Executive Board approved the recommendations for distribution on December 5, 2016.

Based upon the language used in the SAFER Act, the laboratory processing of sexual assault evidence kits, specifically with respect to DNA evidence, is the focus of this document. Sexual assault encompasses all unlawful sexual conduct including, but not limited to, rape, sodomy, incest, child molestation, sex offenses involving minors and other sexual conduct. It is important to note that both genders are victims of sexual assault but for purposes of this document, generalization to a female victim may be made when referring to the tests for male DNA, etc. Also, the following recommendations generally relate to live victims of sexual assaults and there may be special considerations for victims of homicides with a sexual assault component.

As background for these recommendations, considerations, from a laboratory perspective, are provided on the collection of the sexual assault evidence kit for purposes of facilitating the proper collection and storage of DNA evidence. This document also includes a description of various laboratory processes, with a primary focus on sexual assault evidence kits. The advantages and disadvantages of each process will be explained in order to recommend those practices that have been effective in obtaining probative evidence for the timely investigation and prosecution of sexual assault cases. Forensic practices in use in laboratories across the nation are included for illustrative purposes but it is ultimately the responsibility of each individual laboratory to determine what processes and practices will work effectively in its jurisdiction. To the extent that a laboratory wishes to incorporate any of these recommendations, the implementation of any technology or methodology new to that laboratory shall be preceded by the appropriate internal validation to ensure compliance with the *FBI Director's Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories (QAS)*. All

laboratories must have an internal validation that supports their decisions for specific DNA procedures.

A high volume of sexual assault evidence kits can be expected as a result of Federal and State legislative efforts to inventory sexual assault evidence kits and ensure the timely processing of these and future collections of such kits. High throughput processes with increased efficiency and decreased turn around time will be needed to meet this demand and serve the public interest. Laboratories should investigate high throughput methods and implement those that best fit their caseloads and resources. Many aspects are involved in establishing a high throughput process. Even if a laboratory is not able to implement all of the high throughput recommendations, by adopting several key elements in conjunction with some of the alternative approaches, a laboratory should be able to see improvement in its overall efficiency when processing sexual assault evidence kits. A sample flowchart for the high throughput processing of sexual assault evidence kits is contained in Appendix 1.

1. Evidence in Sexual Assault Cases

While most forensic laboratories are government entities, they must remain independent and neutral when evaluating what evidence items to test and methodologies to use. Forensic laboratories should have an evidence submission policy/protocol that includes the prioritization of evidentiary items. Customers and stakeholders (law enforcement, prosecution, defense, etc.) must also take into consideration the resources of the forensic laboratories and thus, exceptions to the evidence submission policy should be a collaborative effort between the laboratory and its stakeholders. Communication between forensic laboratories and their customers/stakeholders is key to achieving success.

The victim,[1] in a sexual assault case, is the crime scene. Sexual assault evidence kits are designed for the collection of evidence from the victim. While the contents of such kits may vary according to each jurisdiction, many kits include a comb, swabs, and containers for the collection of blood, hair, urine and other bodily fluid samples from the victim. In some jurisdictions, the victim's underwear is collected and packaged in the sexual assault evidence kit. For purposes of these recommendations, analysis of the victim's underwear is not included when referring to analysis of the sexual assault evidence kit. The primary focus of this document will be the processing of the swabs submitted as part of the sexual assault evidence kit with the underwear as a secondary option for analysis.

Other Items in Sexual Assault Kit

If the victim's underwear, feminine hygiene items and condoms are included in the sexual assault evidence kit, they should be packaged separately and labeled. Other clothing worn by the victim should be packaged separately from the sexual assault evidence kit and labeled.

Other probative evidence may include evidence collected from the suspect,[2] as well as the victim's and suspect's clothing, and evidence collected from the location where the assault was committed (such as bedding collected from the area of the sexual assault, weapons used in the

[1] This document uses the term *victim*, which is the accepted term used in the criminal justice system, for consistency throughout this document. Professionals should continue to use the terminology (i.e., patient, survivor) appropriate to their discipline.

[2] This document uses the term *suspect*, which is the accepted term used in the criminal justice system, for consistency throughout this document. Professionals should continue to use the terminology (i.e., alleged suspect, assailant, perpetrator) appropriate to their discipline.

commission of the assault, condoms, etc.). These types of items may provide valuable probative evidence of the crime and assist in identifying the suspect; however, they will not be included as the primary focus of these recommendations.

Guidance on the proper storage and preservation of evidence is available in *The Biological Evidence Preservation Handbook: Best Practice For Evidence Handlers*.[3]

1.1 Collection Considerations from a Laboratory's Perspective

Victims of sexual assault may undergo a sexual assault forensic exam conducted by a healthcare professional. During these exams, items of possible evidentiary value are collected and submitted, upon consent of the victim, for the initiation of a criminal complaint and/or investigation. Prior to collection of evidentiary items, healthcare professionals must consider several factors to assist in guiding their collection and treatment efforts.[4] These factors may include the assault activity, time elapsed since the assault, post assault activities, the age and gender of the assault victim, and mental capacity, to name a few. Evidence collection should be guided by the background history, focusing specifically on the suspect's actions during the assault. It is not uncommon however, that as a result of the trauma,[5] victims may not be able to organize the memory of the assault or the actions taken by the suspect. In the absence of a victim able to recollect a complete background history, a full range of samples should be collected assisted by the physical assessement.[6]

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[3] *The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers*, Technical Working Group on Biological Evidence Preservation, NISTIR 7928 (April 2013); available at http://dx.doi.org/10.6028/NIST.IR.7928.

[4] *A National Protocol for Sexual Assault Medical Forensic Examinations* Adults/Adolescents (2d Ed), U. S. Department of Justice, Office on Violence Against Women, NCJ 228119 (April 2013) available at https://www.ncjrs.gov/pdffiles1/ovw/241903.pdf.

[5] *The Neurobiology of Sexual Assault: Implications for First Responders in Law Enforcement, Prosecution, and Victim Advocacy*, Dr. R. Campbell, 12/2012, NCJ 240953, available at https://www.ncjrs.gov/App/Publications/abstract.aspx?ID=263040.

[6] See *A National Protocol for Sexual Assault Medical Forensic Examinations Adult/Adolescent* (2nd Ed.) at pages 87 and 99; "Examiners typically ask patients to provide a medical forensic history after initial medical care for acute problems and before the examination and evidence collection. This history, obtained by asking patients detailed forensic and medical questions related to the assault, is intended to guide the exam, evidence collection, and crime lab analysis of findings." (p. 87); and "Trained examiners should use the medical forensic history and the physical assessment of the patient to guide the evidence collection process." (p. 99).

Additional considerations prior to sample collection must include the activities of the victim following the assault. Activities that may impact evidence collection include bathing, brushing of teeth, mouthwash, vomiting, douching, urination and defecation. Careful consideration of the assault activities and post assault activities prior to sample collection is vital. For example, the analysis of swabs collected by swabbing from areas that are kissed, licked, sucked or bit may be impacted if the victim has showered or bathed between the assault and the time of collection. Internal swabs such as from the vagina, mouth or rectum may still be viable for collection even after showering or bathing by the victim, dependent upon the length and thoroughness of the cleansing and time since the assault. Internal and external swabs should still be collected even if the victim has bathed, as the bathing may not have been vigorous enough to remove the fluids or DNA from the victim. Also, potential biological evidence deposited onto a substrate such as clothing, towels or paper towels do not have the same time restrictions as biological evidence deposited on, or within, the victim's body.[7]

Sexual assault evidence kits (SAKs) are manufactured or purchased as a means to standardize the evidence collection process and ensure the proper packaging of evidence to minimize the potential for contamination and deleterious loss of biological material. Instructional forms typically guide the healthcare professional to prompt the victim for certain background information such as previous consenting sexual acts or allegations of assault. Evidence should be collected once a thorough evaluation of the assault and background history are obtained, if possible. Documentation typically referred to as SAK paperwork contains specific information about the assault, what items were collected during the exam, and personal information from the victim. The SAK paperwork should be included when the sexual assault evidence kit is sent to the laboratory, either inside the kit itself or as an attachment. The details in the SAK paperwork contain important information for laboratory staff to perform many different functions including assessing which items to test, if items are eligible for database entry,[8] and to formulate assumptions used in the interpretation and statistical evaluation of the DNA results.

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[7] See generally, *The Persistence of Seminal Constituents on Panties After Laundering. Significance to Investigations,* R.M. Jobin, M. De Gouffe, Can. Soc. of For. Sci. Jour. 36:1 (2003), available at http://www.tandfonline.com/doi/abs/10.1080/00085030.2003.10757551.

[8] Please see the text box entitled "A Note About Elimination Samples" on page 23 for an explanation of the necessity to collect reference samples from consensual partners.

Evidential items typically collected during the exam may include vaginal or penile[9] swabs, rectal swabs, oral swabs, underwear, exterior body swabs (breast, neck, etc.), fingernail scrapings and/or foreign hair. From the laboratory perspective of recovering as much DNA foreign to the victim as possible during the collection process, measures should be taken to concentrate the foreign material by using the fewest number of swabs necessary for the collection site. To ensure laboratory efficiency, if multiple swabs are used during the collection, they should be collected concurrently and if not concurrently taken, it would be beneficial to note the order of the swabs collected. Based upon current evidence retention policies/law, it is recommended that when more than one swab is collected from an area that these swabs be collected consistently (for example, in the same manner, such as with moistened swabs).

It is also important that buccal swabs or other appropriate reference samples from the victim be collected as well to assist with the interpretation of DNA results. Often mixtures are obtained and the victim's DNA profile may be used to deduce a more informative DNA profile of the suspect for CODIS. Occasionally, sexual assault evidence collection exams may also allow for the opportunity to collect garments of clothing for storage outside of the SAK.[10]

1.2 Reference Sample Collection

Ideally, reference samples will be collected from the victim, consensual partner(s) and suspect(s), if known. Buccal swabs or other appropriate reference samples should be collected to assist with the interpretation of DNA results. Often mixtures are obtained from evidentiary items. Therefore, the victim's DNA profile as well as the consensual partner(s), if indicated in the SAK paperwork, may be used to deduce a more informative DNA profile that can be associated with the suspect. In instances where the suspect has been identified, law enforcement personnel should obtain buccal swabs (reference samples) from the suspect for comparison

[9] As noted previously both genders can be victims of sexual assault.
[10] See generally, Chapter 3 (page 9), *The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers*, Technical Working Group on Biological Evidence Preservation, NISTIR 7928 (April 2013); available at http://dx.doi.org/10.6028/NIST.IR.7928.

purposes. The suspect reference sample must be packaged separately from the SAK and be clearly labeled as a suspect reference sample.

1.3 Evidence Collection Time Frames

Laboratory testing includes locating and characterizing bodily fluids and DNA deposited during the assault to assist with the identification of the suspect of the crime. Although the detection and characterization of body fluids in a forensic laboratory has not changed considerably over time, DNA testing has dramatically increased in sensitivity. The ability to obtain a DNA profile from a suspect has improved, allowing for longer time frames for the collection of biological evidence. It is important for victims of sexual assault to allow collection of evidentiary items promptly. Potential body fluids tested may include blood, semen and/or saliva with the option to collect skin cells left behind for touch DNA. Each body fluid has a different post-coital DNA persistence time with semen having the potential to reside for the longest period of time both on the surface of the body and within a body tract.

Evidence from sexual assault victims should be collected as soon as possible. Table 1 describes recommended time frames for the collection of evidence in sexual assault cases.[11]

Table 1: Recommended Time Frames for Evidence Collection

Type of Assault	Collection Time
Vaginal	Up to 120 hours (5 days)
Anal	Up to 72 hours (3 days)
Oral	Up to 24 hours (1 day)
Bitemarks/saliva on skin	Up to 96 hours (4 days)
Unknown	Collect respective samples within the time frames listed above

[11] References for articles on the persistence of body fluids and DNA collected in sexual assault cases that were used in formulating the recommendations in Table 1 are contained in Appendix 2.

Obtaining a CODIS eligible DNA profile from the DNA foreign to the victim is the objective; however, circumstances surrounding the collection time frame as well as post-assault activities (e.g., bathing/showering) may limit the ability to obtain a full autosomal DNA profile. In the event a CODIS eligible DNA profile is unable to be obtained, partial DNA results and/or Y-STR profiles may also provide investigative information.

Sexual assault evidence should be collected from a victim as soon as possible and up to five (5) days or longer post vaginal assault.[12] Due to advancements in DNA technology, emerging research indicates there may be potential to extend the time frame to nine (9) days post vaginal assault.[13] Rectal assaults should result in collection up to three (3) days post assault. Evidence in an oral assault will remain for a significantly shorter period of time and therefore should be collected as soon as possible, but not more than 24 hours from the time of the assault. Alleged assaults that may have resulted in deposition of semen externally (victim clothing, bedding, etc.) should also result in evidence collection because semen will remain indefinitely on these items as long as they are unwashed. Even if items associated with the assault have been washed, they should still be collected as studies have shown that semen can remain on items and continue to be detected even after the items are washed.

A backlog is defined as a case(s) received by the laboratory that exceeds the laboratory's capacity and is (are) awaiting testing.

2. Laboratory Processing of Sexual Assault Evidence Kits

Many factors may impact the ability of a laboratory to process sexual assault evidence kits in an efficient and timely manner. Some of these factors include a backlog of pending evidence/cases, availability of automation, personnel, robotics and other resources.

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[12] While time frames for sample collection tend to fall between 96 and 120 hours in the majority of jurisdictions, the Department of Defense currently extends their collection period to 7 days (Victim Instructions, DD2911, p. 1). See also, *National Protocol for Sexual Assault Medical-Forensic Examinations Adult/Adolescent,* 2nd Ed. at page 8, available at https://safetasource.site-ym.com/resource/resmgr/Protocol_documents/SAFE_PROTOCOL_2012-508.pdf. SWGDAM Guidelines for the Processing of Sexual Assault Evidence Kits in a Laboratory.

[13] P. Speck, J. Ballantyne, *Post Coital DNA Recovery*, December 2014: NIJ Grant No. 2009-DN-BX-0023; available at https://www.ncjrs.gov/pdffiles1/nij/grants/248682.pdf.

While jurisdictions may vary in their definition of a backlog based on legislation or policy, it is generally considered as a case received by the laboratory that exceeds the laboratory's capacity and is (are) awaiting testing. Spurred by victim advocates and legislative mandates, efforts are underway in many jurisdictions to identify and inventory the number of sexual assault evidence kits that have not yet been submitted for analysis. For example, legislation/policy in the following states (Arkansas, Colorado, Delaware, District of Columbia, Florida, Georgia, Hawaii, Idaho, Iowa, Illinois, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Ohio, Oregon, Pennsylvania, Tennessee, Texas, Virginia, Washington and Wisconsin) requires that their law enforcement agencies inventory and report on the number of sexual assault evidence kits that have not been submitted to a laboratory for analysis. As a result of these inventories, laboratories may be faced with submissions of sexual assault evidence kits needing analysis that exceed their current capacity (backlogs).

Laboratories may employ a variety of strategies to address the analysis of SAKs including instituting evidence submission policies, employing high throughput processing and/or seeking additional resources to expand capacity, or outsourcing. Outsourcing is not a long term solution to address laboratory capacity but it may be able to provide laboratories with additional capability to assist with a backlog of SAKs. It is essential that a laboratory or law enforcement agency that chooses to use outsourcing ensure compliance with the FBI Director's Quality Assurance Standards (QAS) on outsourcing. In keeping with the objective of generating a CODIS eligible DNA profile, the vendor laboratory must be accredited by an approved accrediting agency and comply with the QAS in generating the DNA records. A checklist for law enforcement agencies that are considering outsourcing is provided in Appendix 3 to ensure that the DNA profiles generated by the vendor laboratory will be eligible for CODIS.

Laboratories may also consider instituting evidence submission policies that limit the items that will be analyzed in order to streamline the analytical process. For example, analysis of the swabs contained in the sexual assault evidence kit is recommended as a first tier approach for

generating a DNA profile. Then, as needed, a secondary option would be analysis of the victim's underwear.

All laboratories should also consider the volume of sexual assault cases they handle to review their input/output, identify where bottlenecks occur, and determine if a high throughput approach to processing will achieve efficiencies. Laboratories may benefit from the use of business process improvement tools[14] to track the path of sexual assault evidence kits first to understand and document the process flow of this evidence, the laboratory capacity and the staff assigned to each step or task. In performing this exercise, deficiencies or holdups in the processing of these evidence kits will be identified for improvement. By understanding and documenting the process and existing staff allocations/capacity, the laboratory will be in a better position to determine the points in the process and/or staffing that could be improved. The following recommendations, separately or in combination, are designed to assist a laboratory achieve a more efficient processing of sexual assault evidence kits and ultimately, reduce the turn around time for the analysis of the sexual assault evidence kits.

2.1 High Throughput Processing of Sexual Assault Evidence Kits

The high throughput processing concept relies on the fact that most sexual assault evidence kits contain a single common collection substrate of swabbings from various body orifices. Since serological examinations are performed on each piece of evidence individually, it is not amenable to high throughput processing of the SAKs. High throughput processing is a standardized approach that includes laboratory optimization to maximize the development of evidentiary DNA profiles using the following: Laboratory Information Management System

[14] Generally, business process improvement tools are designed to assist an organization to understand its current operations and identify areas for change or enhancement. Business process improvement includes process mapping, root cause analysis and visual management. An organization can use charts, flowcharts, or other tools to track how it operates. For example, process mapping is one such tool and is described as "a structural analysis of a process flow (such as an order-to-delivery cycle), by distinguishing how work is actually done from how it should be done, and what functions a system should perform from how the system is built to perform those functions. In this technique, main activities, information flows, interconnections, and measures are depicted as a collage on a large sheet of (commonly brown) paper, with different colored 'Post-it' notes or slips of paper. This graphic representation allows an observer to 'walk through' the whole process and see it in its entirety." (BusinessDictionary.com).

(Section 2.1.1); standardized case approach with consistent sample types (Section 2.1.2); targeted testing approach (*Direct to DNA*) (Section 2.1.3); defined work flow (Section 2.1.4); automated technologies and robotic instrumentation (Section 2.1.5); interpretation and uniform reporting (Section 2.1.6); and dedicated personnel and resources (Section 2.1.7).

High Throughput Processing has the following components:

LIMS
Standard case approach
Targeted testing
Defined workflow
Automation
Uniform reporting
Dedicated resources

2.1.1 Laboratory Information Management System (LIMS)

A Laboratory Information Management System (LIMS) is able to maintain and track evidence and samples as they move throughout the laboratory. A LIMS will allow for the ability to store information about a case in an electronic manner and will record transfers of evidence. A LIMS could also be used to generate reports for the laboratory and to track basic metrics to evaluate, assess and continuously improve laboratory processes.

A LIMS is recommended for any laboratory processing a high volume of samples because it is able to provide improved documentation, accuracy and speed. A fully integrated LIMS system will gather information from various sources and move that input seamlessly through the process, including generation of the final report, electronic report distribution, case scheduling and prioritization around court dates, as well as various output and flow reports. This system may include data from the submitting agency, instrument and robotic output, quality control, sample tracking, and case status. The use of a LIMS results in analyst time saved and an increase in data quality and quantity.

It is also helpful to have a sample tracking mechanism to identify samples as they are processed. An efficient way to accomplish the tracking is through the barcoding of evidence and samples which can be managed through a LIMS. Barcoding of samples also helps to eliminate the potential for error in switching the samples. The barcoded samples can be linked to the chain of custody verifying that they are moved throughout the process and tracked along the way.
A LIMS can be developed internally within the laboratory or purchased through a commercial vendor. Either option will be a major investment for a laboratory. A LIMS will require training, validation, ongoing maintenance costs, security and back-up plans, and specialized information technology (IT) technical support.

2.1.2 Standardized Case Approach with Consistent Sample Types

As mentioned previously, most SAKs contain a single common collection substrate of swabbings from various body orifices. By developing a laboratory approach to capitalize on this uniform substrate, the variability is removed and more emphasis can be placed on the method behind which the necessary genetic material from those samples is obtained for DNA processing.

From a high throughput perspective, time can be gained by processing all of the evidentiary swabs provided in the sexual assault evidence kit with the *Direct to DNA* process. Testing all swabs lends itself to consistency within a laboratory among multiple analysts, which helps to standardize case approach by removing subjective decision points. This approach is also recommended for cases in which the victim was incapacitated or cannot describe the assault. Due to the lack of information about the assault, all evidentiary swabs should be tested in order to gain as much information about the assault as possible. The limitation on the testing of all swabs is that it leads to an increase in the amount of data generated that must be analyzed, interpreted, reviewed and reported. Before a laboratory decides to process all swabs in all sexual assault cases, consideration should be given to how the laboratory will address the influx of data.

2.1.3 Targeted testing approach: *Direct to DNA*

An integral component of high throughput processing is to proceed directly to DNA (also known as the *Direct to DNA* approach) and then, as needed, follow up with serology, which requires the

most hands-on time from an analyst. The serology tests employed by laboratories are less sensitive than modern DNA typing kits. Therefore, DNA typing only the swabs that screen positive in serology tests may miss CODIS-eligible DNA profiles. Rather than use serology to determine which swabs to subject to DNA analysis, a *Direct to DNA* approach is recommended. In the *Direct to DNA* approach, DNA analysis is performed before serology to maximize the chances of obtaining CODIS eligible profiles. This approach has the added benefit of being more efficient than performing serology followed by DNA analysis. As long as a portion of the swabs are retained, serology can be performed, as needed, after DNA testing is complete. Thus, the *Direct to DNA* approach allows the laboratory to analyze the evidence and obtain information necessary to search CODIS in a more timely manner.

With the increase in sensitivity of the commercially available DNA kits, the *Direct to DNA* approach uses the most sensitive technique first and may be followed by, only when necessary, serological examinations and/or Y-STRs at a later time. Processing the swabs from a SAK for DNA first allows for potential reduction in false negative serology results of swabs that are not moved on for DNA analysis because they fell below the serological test's limit of detection. When a laboratory uses a *Direct to DNA* approach to processing the kits, the following issues must be addressed:

- ✓ Are all swabs going to be tested? If not, which swabs will be targeted for testing?
- ✓ How much of each swab will be used for DNA testing?
- ✓ Will a male DNA screening approach be used?
- ✓ What kit will be used for quantification? Does the internal validation permit discontinuing analysis based on the quantification value?
- ✓ What kit(s) will be used for amplification?
- ✓ What instrumentation will be used to help increase laboratory efficiency?
- ✓ Has mixture software been considered to help the analysts interpret the data?

Similar to the processing of known reference samples (typically collected from cheek swabbings from individuals used for comparison purposes), swabs from the sexual assault evidence kit would not be screened for biological fluids and would move straight into DNA extraction. Automation is key to ensuring this process maintains a high throughput method and does not create a backlog in itself.

Forensic samples can be divided into two categories based on the type of DNA extraction that would be performed. The forensic samples can be separated into a differential extraction (separating the epithelial cells from sperm cells) or a non-differential extraction (lysing all cells at once) category. Swabs in a sexual assault evidence kit require a differential extraction if semen is expected to be present. Other swabs, believed to contain blood, saliva or skin cells based on the scenario descriptions, are suitable for a non-differential extraction. Initial decisions required by the laboratory include which swabs in the sexual assault evidence kit to carry through the DNA process or potentially analyze all samples. The approach to cut all swabs for DNA processing is only a viable option if the laboratory uses automated methodologies for extraction and robotics to set up the quantification and amplification due to the number of samples that would be carried through at any one time. If the laboratory decides to process all swabbings from the sexual assault evidence kit, it would be impractical to take every single swab through processing individually. Combining specific swabs taken from the same orifice is important in order to concentrate the sample and maximize the detection of foreign DNA. In the approach of testing all swabbings, some triage of samples requiring differential extraction as opposed to non-differential extraction should occur, although it adds a level of decision making that may not be conducive to a high throughput method or standardization.

If the approach that the laboratory chooses is to continue with all swabs through extraction, a portion of each swab should be taken while a portion may be left behind for potential retesting. If multiple swabs from the same area are collected, those may be combined

A Consideration for the Identification of Sperm

With a *Direct to DNA* approach, a laboratory should establish a policy regarding the identification of sperm. A laboratory may choose to bypass the identification of sperm or choose to identify sperm on a case-by-case basis. If the confirmation of sperm is determined to be necessary, a laboratory may consider preparing a microscope slide during the differential extraction process prior to sperm lysis. Alternatively, a laboratory may choose to re-examine a swab for the presence of sperm following the completion of all DNA testing by re-sampling the remaining swab. This approach would be recommended for laboratories that choose to identify sperm only as needed on a case-by-case basis. This process would improve efficiency by eliminating the time required to prepare microscope slides during the extraction process.

during the extraction step and can be concentrated in order to obtain as much DNA as possible from the samples without proceeding with too many samples.[15]

The extracted DNA can be quantified using appropriate and validated procedures with a robotic liquid handling system to pipette and prepare samples and reagents into plate format. At this point the laboratory can employ validated quantification thresholds and/or quantification ratios to decrease the number of samples in a set to be amplified and/or make decisions about which amplification kit will be used.

2.1.4 Defined Work Flow Examples

2.1.4.1 Screening for Male DNA via Quantification

If a laboratory chooses to start the *Direct to DNA* approach by screening for male DNA in each sample, the decision of how much sample may be consumed must be evaluated first. This type of approach replaces the typical serological testing with a more sensitive DNA-based technique. Experience and data have shown that the quantification and Y-STR kits currently available are significantly more sensitive than any screening test performed in serology.

A quick non-differential extraction technique that uses a very small portion of one swab (~ 1/8 to 1/16) can be performed followed by a quantification method that detects both human and male DNA to determine if male DNA is present. If no male DNA is detected then no further processing of that sample may be necessary. If male DNA is detected at the quantification screen stage, then a technician or analyst may return to that sample and remove a larger portion of the swab to be taken through a high throughput process to include differential (if applicable) extraction, quantification and amplification using automation and robotic instruments for plate set-up. Depending on the capabilities of the laboratory, Y-STR testing may be pursued on

[15] For example, if four vaginal swabs were collected, cut at least ½ of two swabs and place into one tube for extraction while cutting ½ of the other two swabs into a separate extraction tube. Following a differential extraction, the sperm fractions would be combined and the epithelial fractions would be combined for a total of 2 tubes to move on to quantification. Depending on the elution volume from the extraction and combination, the laboratory may choose to dry down samples, through the use of a speed vac or other process, to concentrate the samples to appropriate levels. The concentration step would be more appropriate for sperm fractions but may not be helpful for the epithelial fractions because the epithelial fraction may already contain high levels of victim DNA.

samples with very low levels of male DNA, which might not otherwise provide useful results with autosomal DNA testing. Laboratories must perform internal validation studies to determine their quantification thresholds.

Laboratories can save time and resources if their quantification method is sensitive and specific enough to justify ceasing analysis on a sample that results in either no human DNA and/or no male DNA. Additionally, this quantification can be used to select which samples to move through the DNA process. If there is only one suspect, it may not be necessary to proceed with multiple swabs.

2.1.4.2 Microscopic Identification of Sperm

With a *Direct to DNA* approach, a laboratory should establish a policy regarding the identification of sperm. A laboratory may choose to bypass the identification of sperm or choose to identify sperm on a case-by-case basis. If the identification of sperm is necessary, slides prepared with an aliquot of sperm fraction from the differential extraction are generally easier to view than slides prepared by the healthcare professional during the exam because they have less epithelial cells, bacteria, and other debris. Preparation of slides in this manner improves efficiency by eliminating other forms of serology without sacrificing a sperm identification that could potentially be needed at trial. These slides may be stained and viewed to confirm the presence of sperm. Alternatively, the slides can be spotted, dried, preserved, and returned with the evidence to be stained and viewed at a later time, if necessary.

2.1.4.3 Quantification and Amplification

The newer quantification kits being manufactured have an increased sensitivity and several laboratories have used these kits to determine a threshold level for moving forward with amplification. As a result, laboratories should review the kit(s) currently online to evaluate if their quantification method is sensitive and specific enough to justify ceasing analysis on a sample that returns either no DNA at all, or no male DNA, depending upon the circumstances. For laboratories with an established threshold, samples that fall below that threshold do not need to be amplified. Establishing a decision point where laboratories can cease analysis on negative cases early in the process can be critical to the success of high volume throughput. Therefore,

implementing a quantification system that provides a true negative when a profile cannot be obtained in subsequent analysis should be a high priority.

Amplification kits are now much more discriminating based on the number of autosomal loci they contain as well as incorporating several male-only locations. In most cases, moving forward with an autosomal amplification typing kit will provide the most relevant information, especially if the subject is not known and a DNA profile foreign to the victim is generated. The objective under these circumstances is to be able to generate a DNA profile that can be searched in CODIS, even if a full profile is not obtained.

2.1.5 Automated Technologies and Robotic Instrumentation

Automation is key to ensuring this process maintains a high throughput method and does not create a backlog in itself. Automation and robotic instrumentation is designed to achieve higher output, increase accuracy, allow multiple tasks to be performed at the same time (by the instrument and analyst) and maximize the use of laboratory personnel. A manual method would not allow an analyst or technician to be able to process as many samples in a day as a robotic instrument. Robots or automated instrumentation allows the analyst to set up for the process then walk away to complete other tasks while the instrument performs the appropriate step of the process. Automation may allow the analyst more time to work on interpretation and data analysis, thereby improving the response or turn around time.

Incorporation of robotics or automation at each step of the DNA process will provide the most efficient high throughput approach. Laboratories should consider the optimal size and construction of sample groups or batches to reduce bottlenecks and increase sample throughput. Incorporating robots as liquid handling systems to handle laboratory processes like quantification and amplification into a plate format provides for more efficient preparation while ensuring more reproducible pipetting of potentially small volumes. Using a robotic platform to handle sample preparation into plate format increases the overall quality of laboratory processes by reducing transfer error or switches.

2.1.6 Interpretation and Uniform Reporting

Depending on the decisions about which samples to process, the laboratory may find itself with an abundance of DNA profiles, which leads to more analysis, more interpretation, more mixtures, and potentially difficult statistical reporting. An approach to streamline the results and interpretation is through the use of standardized reporting templates. A paperless system facilitated by an integrated LIMS may help to alleviate a portion of the administrative burden. Specialized software may also be employed to assist in the interpretation of mixed DNA profiles.

The laboratory should have and follow written procedures for taking and maintaining case notes to support the conclusions drawn in reports. The laboratory should maintain all analytical documentation generated by the technicians or analysts related to the testing. The laboratory should retain, in hard copy or electronic format, sufficient documentation for each technical analysis to support the reported conclusions such that another qualified individual could evaluate and interpret the test results. All laboratories should follow the *QAS* and relevant accreditation requirements when reporting and reviewing results.

Where a *Direct to DNA* approach is adopted, the DNA results from the items analyzed in the initial round of testing can be reported in a streamlined report that addresses the following criteria:

1. Was male DNA detected on any of the items?
2. Was an unknown DNA profile generated?
3. Was the unknown DNA profile uploaded to CODIS?

Using such a template format can provide the investigative information obtained through searches of the database more quickly and simply. Quantitative statements and detailed tables showing alleles are not necessary in such a streamlined report. Other information required by the *QAS*, such as technologies used and loci tested, can be included in a standardized statement or appendix.

When serological examination of items is performed, for example at the time of submission of a relevant comparison sample, notification of a database hit, or upon special request, an additional or supplemental report must be sent to the submitting agency.

Reference samples from the victim, suspect and any other elimination known samples (e.g., consensual partner) are compared to the data in the case and the appropriate statistics will be applied. Systems that provide an IT component can ease the complex statistical calculations needed. Probabilistic genotyping software may aid in the evaluation of more complex interpretations, which may result in decreasing the time necessary to conduct manual deconvolutions for mixtures. Specialized software will provide increased consistency and make better use of the data in which profile comparisons and statistical calculations are made.

2.1.7 Dedicated Personnel and Resources: Organization and Staffing

An organized approach on how to best handle the caseload in a laboratory requires the laboratory to review its input and output to identify where bottlenecks occur. As noted previously, business process improvement tools should be utilized which include process mapping, root cause analysis and visual management. Process mapping is one component of the business process improvement and is an exercise designed to chart the flow of the evidentiary items and staffing allocations through the laboratory providing a complete picture of how the laboratory handles the sexual assault evidence kit. Using the information obtained through the process mapping exercise, the laboratory could dedicate appropriately trained staff to specific tasks when the need exists to eliminate a potential buildup of cases through optimization of process flow. Visual management also helps a laboratory to quickly identify bottlenecks in the process. Many laboratories have implemented a system of batching samples in the laboratory due to the automation available. Staff may also be organized into teams to enhance communication and throughput. This approach could potentially free up analyst time in order to focus on interpretation and reporting.

Additionally, undergoing business process improvement can identify the laboratory's physical organization of tasks. These exercises may identify taskings and work spaces that could be modified to enhance work flow and minimize steps.

3. Turn Around Times

Generally, a backlog in sexual assault evidence kits will impact the laboratory's overall turn around time for the processing of a sexual assault evidence kit because a backlog may lead to delays or bottlenecks in the inventory, assessment, processing and analytical tasks within the laboratory. The existence of a backlog in the laboratory will impact the ability to achieve the ideal turn around times. While efficiencies may be achieved in the analytical process through implementation of one or more of the recommendations (LIMS, standard case approach, *Direct to DNA*, defined workflow, automation, uniform reporting, or dedicated resources), a laboratory's capacity will continue to influence overall turn around time for the processing of the sexual assault evidence kit.

Several jurisdictions have instituted, through legislation, turn around times for the analysis of sexual assault evidence kits, once received in the laboratory. These time frames range from 60 days to 6 months for the processing of the sexual assault evidence kit. Table 2 contains a sample of State legislation instituting turn around times for the analysis of sexual assault evidence kits.

Consistent with the intent of these State legislative initiatives and for purposes of these recommendations, turn around time is the period of time between the receipt of the sexual assault evidence kit in the laboratory and all relevant testing of the kit is complete, eligible DNA profiles are uploaded to CODIS, and the report issued.

Table 2 – Sampling of State Legislation on Laboratory Analysis of SAKs

State	Turn Around Time for Laboratory Analysis of SAKs	Reference
California	The crime lab should do one of the following for any sexual assault forensic evidence received by the crime lab on or after January 1, 2016: Process sexual assault forensic evidence, create DNA profiles when able, and upload qualifying DNA profiles into CODIS as soon as practically possible but no later than **120** days after initially receiving the evidence; or Transmit the sexual assault forensic evidence to another crime lab as soon as practically possible, but no later than 30 days after initially receiving the evidence…	CA Penal Code §680(b)(7)(B)(i); see also CA Penal Code §803(g)
Colorado	Upon submission to an accredited crime laboratory, that laboratory must strive to analyze and, when appropriate, upload the information into CODIS within six (6) months of receipt of the forensic medical evidence being submitted, assuming the laboratory has sufficient resources.	Colo. Rev. Stat. §24-33.5-113 ; 8 CCR 1507-29
Connecticut	If the evidence is transferred to the division, the division shall analyze the evidence **not later than sixty days** after the collection of the evidence… If a victim reports the sexual assault to the police department after the collection of the evidence, such police department shall notify the division that a report has been filed not later than five days after filing such report and the division shall analyze the evidence **not later than sixty days** after receiving such notification.	CT Gen. Stat. §19a-112a(d)
District of Columbia	The DFS (Department of Forensic Sciences) shall process all sexual assault forensic examination kits **within 90 days** from the date of receipt.	D.C. Code §4-561.02(b)
Florida	Effective July 1, 2016 Testing of sexual offense evidence kits must be completed **no later than 120 days after submission** to a member of the statewide criminal analysis laboratory system.	Florida Statutes §943.326 (4)
Idaho	For all sexual assault evidence kits received pursuant to this section, the Idaho State Police Forensic Services Laboratory shall test such kits and submit eligible results to the Idaho DNA database **within 90 days**.	Idaho Code §67-2919(4)
Illinois	All sexual assault evidence submitted pursuant to Section 10 of this Act [submitted after September 1, 2010]….shall be analyzed **within 6 months after receipt** of all necessary evidence and standards by the State Police Forensic Laboratory or other designated laboratory if sufficient staffing and resources are available.	725 ILCS §202/10.
Kentucky	The department shall analyze and classify all sexual assault evidence collection kits it receives. In cases where a suspect has been identified, the department may give priority to analysis and classification of sexual assault evidence collection kits where the reference standard for comparison is provided with the kit. Except as provided in paragraph (e) of this subsection [if appropriated funds are insufficient], by July 1, 2018, the average completion rate for this analysis and classification **shall not exceed 90 days** and by July 1, 2020, the average completion rate **shall not exceed 60 days.**	Kentucky Rev. Stat. §17.175(3)(a)
Michigan	All sexual assault kit evidence submitted to the department or an accredited laboratory on or after the effective date of this act shall be analyzed within **90** days after all necessary evidence is received by the department or other accredited laboratory, provided that sufficient staffing and resources are available to do so.	Mich.Rev. Stat. §752.934(6)
Ohio	Perform a DNA analysis of the sexual assault examination kit **as soon as possible** after receiving the kit (and enter the resulting DNA record into a DNA database).	Ohio Rev. Code §2933.82(B)(2)(d)(i)
Pennsylvania	A laboratory shall complete the testing or analysis of all sexual assault evidence submitted pursuant to this section within **six months from the date of receipt** of the evidence, if possible.	35 P.A. §10172.3(c)(4)
Texas	If sufficient resources and staffing are available, a public accredited crime laboratory **as soon as practicable** shall complete its analysis of sexual assault evidence submitted under this chapter or other law.	Texas Gov't. Code §420.042

The recommendations for high throughput processing using a *Direct to DNA* approach may allow a laboratory to achieve a 2-4 week turn around time for the analysis of SAKs under the defined ideal circumstances of all of the following:

- ✓ Little or no backlog;
- ✓ Implementation of a *Direct to DNA* approach;
- ✓ The use of automation;
- ✓ Appropriately trained and dedicated staff;
- ✓ A laboratory information management system (LIMS);
- ✓ Automated methodology (extraction, quantification, and amplification) with the use of robotic instruments;
- ✓ Sufficient resources for personnel, instrumentation, and consumables; and
- ✓ The use of key decision points to determine the appropriate items to process in each SAK.

4. Combined DNA Index System (CODIS)

CODIS is the system of DNA databases at the national (NDIS), State (SDIS), and Local (LDIS) levels for storing and searching DNA records contributed by Federal, State and Local forensic laboratories for law enforcement identification purposes. An important objective in analyzing the sexual assault evidence kit is to generate a CODIS eligible DNA record that can be uploaded into CODIS and the National DNA Index System (NDIS). The Federal DNA Identification Act ["Federal DNA Act"; 42 U.S.C. §14132] governs the National DNA Index System which is administered by the Director of the Federal Bureau of Investigation. The Federal DNA Act authorizes the inclusion of DNA records in NDIS from "analyses of DNA samples recovered from crime scenes" known as forensic DNA records. Every State participates in the national level (National DNA Index System) and contributes DNA records of designated offenders, arrestees and/or forensic records. Pursuant to the Federal DNA Act, only criminal justice agencies may participate in the National DNA Index System.

4.1 CODIS/NDIS Eligibility

In determining the eligibility of forensic DNA profiles for CODIS and NDIS, there should be documentation that a crime has been committed and the evidence has been obtained from the crime scene. The crime scene (forensic) indexes at NDIS contain DNA profiles from forensic samples recovered directly from the victim (such as a SAK), the victim's clothing, or the crime scene, and are believed to be attributable to the suspect.[16] Accordingly, in cases without known suspects, ensuring eligibility for uploading the profile foreign to the victim is key. If a consensual partner is listed, the laboratory must request that an elimination reference sample be submitted for comparison purposes prior to entering the DNA

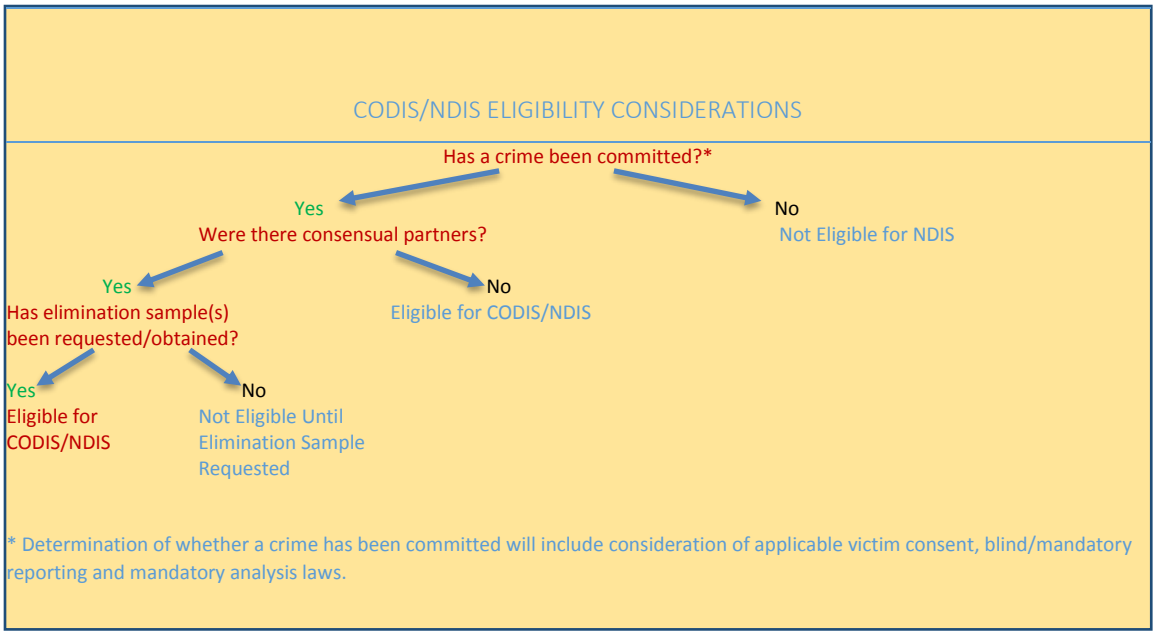
A Note about Elimination Samples

Elimination samples are needed in a sexual assault case when the victim indicates that he/she engaged in consensual sexual relations in close proximity to the occurrence of the sexual assault. In those instances, law enforcement officials should request and document consent for a DNA sample from the consensual partner in order to eliminate that partner's DNA profile from consideration as the forensic unknown(s) developed from the sexual assault evidence kit. And, if the elimination sample obtained from a consensual partner is matched to the sexual assault evidence kit, that forensic DNA record must be removed from CODIS and NDIS in accordance with the Federal DNA Act [42 U.S.C. §14132(a)(1)(C)].

[16] NDIS Operational Procedures Manual, Section 3.1.1.1, (2016) Federal Bureau of Investigation Laboratory, available at https://www.fbi.gov/file-repository/ndis-procedures-manual-ver4-approved-04272016.pdf/view.

profile generated. Elimination profiles are compared to the foreign DNA profile and if they match, that foreign profile is not eligible for CODIS. Elimination profiles can also be used to determine what is foreign to both the victim and consensual partner (elimination) for possible upload to CODIS.

Considerations for CODIS/NDIS Eligibility



If a hit occurs in CODIS, the name of the individual will be released to the law enforcement agency by the laboratory once the internal confirmation process is completed. It is then up to the law enforcement agency to collect a reference sample from the suspect and submit it to the laboratory for DNA testing. It is this analysis of the suspect's DNA and comparison with the evidentiary sample that will provide the evidence for court. Hit tracking software can be used to electronically distribute hit reports; monitor the receipt, action and outcomes of hits; provide data on trends; and permit follow-up.

The DNA profiles relating to the unsolved and solved crimes remain in the Local, State and/or National DNA databases for future searches and potential hits.

5. Secondary Testing

If a profile is generated that is eligible to be uploaded into CODIS, or is able to be matched to a suspect, then no secondary testing may be necessary. Case scenarios should always be considered in the event of multiple suspects, consensual partners, or other information that is needed to maximize evidentiary potential.

5.1 Y-STR Testing

Y-STR analysis can be useful in situations when the information gained from autosomal DNA analysis is negligible or non-existent, yet trace male contribution is detected. By testing genetic loci limited to the Y-chromosome, competition and masking from female DNA is no longer a factor. Cases shown to benefit from this selective amplification include extreme female:male mixtures (e.g., low sperm counts on vaginal samples, female's fingernail clippings) and for the possible resolution of male:male mixtures (e.g., major and minor contributors). It is important to note, however, that a Y-chromosome DNA profile is shared by an individual's father and all his patrilineal relatives and is therefore not as discriminating as autosomal results and is not eligible for searching in NDIS. Commercially available Y-STR kits allow for the analysis of up to 27 loci and allow an evidence-to-known comparison to be conducted. Suspect or elimination reference samples analyzed with Y-STRS may provide investigative information in the absence of a CODIS eligible profile.

5.2 Serological Testing

Historically, serological testing of body fluid detection and identification has been performed prior to DNA analysis. While this approach either indicates or confirms which body fluids are on the evidence, some of these testing techniques are prone to cross-reactivity as well as sample/time consumption. The laboratory is encouraged to weigh the information gained from this body fluid testing as compared to time and sample saved by going *Directly to DNA* analysis. As the sensitivity of DNA analysis has increased beyond that of serological screening techniques, there is also the consideration that DNA profiles could be developed from biological sources that may not be detected by serology. By processing samples using DNA technology prior to serological testing, it eliminates the possibility of obtaining false negative results due to the limit of detection for the serology tests. DNA is being detected in samples that may not have

been previously analyzed. Whether all swabs are processed or only select swabbings are tested, DNA analysis can begin immediately instead of being delayed by sperm searching or other body fluid testing.

5.2.1 Targeted Serological Testing

If serology is necessary for a case, it is recommended to use a targeted approach to most efficiently process sexual assault evidence kits. Such a targeted approach would focus on the needs of a specific case; for example, a request to identify sperm in the assault of a child victim.

Semen, blood and saliva testing methods (AP, Phenolphthalein, TMB, immunological tests for blood, etc...) consume sample and may be subject to cross-reactivity. These types of serological based testing do not employ the most sensitive techniques available, however, some case circumstances do necessitate the need that blood, semen or saliva be presumptively present or confirmed. For example, it may be necessary to determine if the male contribution from an evidentiary swab obtained from the victim is presumptive for saliva or semen. Alternatively, in cases involving children, blood testing may provide important investigative information.

Presumptive tests are designed to be sensitive but not necessarily specific, meaning that cross-reactivity could occur. Confirmatory tests have a high specificity, but are not always the most sensitive of testing procedures. Any serology test consumes valuable sample that could be used for more informative DNA analysis and these serological techniques should be reserved for the triage of larger evidentiary DNA items and items with a significant amount of biological material. All presumptive or confirmatory testing should be conducted in the forensic laboratory and not during the sample collection process at the time of the medical-forensic exam.

5.2.2 Presumptive and Confirmatory Methods of Testing

5.2.2.1 Acid Phosphatase (AP) Semen Test

Testing for the presence of acid phosphatase (AP) is a colorimetric test based on the reactivity of the acid phosphatase enzyme found in high concentrations in semen, but its presence is not limited to semen only. AP can be detected in lower levels in other body fluids such as vaginal fluid as well as in bacteria and white blood cells common to the female genital tract.

When used for semen detection in sexual assault evidence kits, it can be an indicator of the presence of seminal fluid and act as a guide for how much of the swab(s) to consume for DNA analysis. A strong positive reaction in the absence of spermatozoa may be an indicator of an aspermic, azospermic or vasectomized suspect indicating the probative DNA evidence may be found in the non-sperm fraction (or that a differential extraction may not be warranted). These same concepts apply to the use of AP testing on a pair of underwear; however, this testing may help to triage multiple stains and/or map areas of possible semen on the evidence.

5.2.2.2 Prostate Specific Antigen (P30) Semen Test

Historically, laboratories treated p30 as a confirmatory test for the presence of seminal fluid, but some laboratories now consider it a presumptive test for semen. If using as a confirmatory test, laboratories should ensure that naturally occurring p30 in other substances (vaginal fluid, urine, blood, tears, breast milk, etc.) will not react and give a positive result. However, a positive p30 test along with a positive AP test is strong evidence of the presence of seminal fluid as opposed to other body fluids, which would give negative results to both tests. The immunological test itself consumes some sample and takes incubation time.

5.2.2.3 Microscopic Sperm Searching

It is recommended that if identification of sperm is conducted, that samples for this purpose are made by the laboratory. Either an extract of the swabs (or material) can be made using traditional means, or a portion of the sperm-fraction prior to sperm lysis can be spotted on a microscope slide. Both methods result in more efficient sperm searching by providing a smaller, more discrete area for microscopic examination. The latter provides a much cleaner extract without epithelial or bacterial cells.

If the identification of sperm is deemed necessary for trial purposes, the laboratory has options to consider in its workflow. Slides can be viewed to confirm the presence of sperm or they can be spotted, dried, preserved, and returned with the evidence to possibly be stained and viewed at a later time. Alternatively, if necessary for trial, a laboratory may reexamine a swab for the

presence of sperm following the completion of all DNA testing by resampling the remaining swab.

5.2.2.4 Blood Testing

The necessity of testing for the presence of blood on swabs or underwear in a sexual assault evidence kit is case and victim dependent. Depending on the method of blood testing selected, it can either be considered a presumptive or confirmatory test. Sample consumption and time to conduct the tests are proportional to the information gain. The confirmatory test is conducted with a larger amount of sample and takes longer to perform than the presumptive test.

5.2.2.5 Visual Examinations

Examination of evidence items using an alternate light source (ALS) may aid in locating body fluid stains such as semen; however, the presence of any fluorescence from a dried stain is not a conclusive identification of what, if any, biological fluid may be present on an item of evidence. A stain must be tested chemically if any serological conclusions are to be made concerning the type of body fluid present.

If it is necessary to examine a pair of underwear, biological evidence should not be the only type of evidence to be considered. Hairs, fibers, and/or fabric damage (cuts, tears or seam separations) may also be probative to the case. Hairs with roots should be assessed for DNA suitability.

6. Outsourcing

Forensic laboratories are already operating at capacity with current caseloads covering all types of cases; however, the SAFER Act of 2013 has highlighted the issue of sexual assault evidence kits that were never submitted to a laboratory for analysis. Regardless of the reason(s) for non-submission to the laboratory at the time of the crime, this attention has resulted in the identification of thousands of these kits for analysis – both for historical and current cases; requiring forensic laboratories to reexamine how these cases are analyzed in order to provide a more timely public service. A common (and historical) reaction to such an influx of cases is to outsource; however, experience shows that this is not a long term solution and may create a significant amount of work for the originating laboratory with the inventorying and tracking of

kits, quality control samples, subsequent CODIS reviews and uploads of appropriate cases. Additionally, this approach does not enhance the laboratory's infrastructure by building capacity to process an increase in cases.

If outsourcing is used, it is important to ensure the vendor laboratory's compliance with the accreditation and *QAS* requirements of Federal law so that the DNA records generated by the vendor laboratory are eligible for upload to CODIS:[17]

- ✓ Compliance with the *FBI Director's Quality Assurance Standards (QAS)*, in particular the provisions on outsourcing; and
- ✓ Accreditation by an approved accrediting agency.

An agency that stores and searches its vendor laboratory generated DNA records in a local DNA database that is not part of the CODIS system operates such a local DNA database according to local policies for the collection and use of these DNA samples and records. Because a non-CODIS local DNA database operates in accordance with local policies and procedures and not the Federal DNA Act requirements and protections, such a non-CODIS local DNA database cannot be a participant in NDIS nor does such a non-CODIS local DNA database have access to NDIS or the DNA records in NDIS. Thus, the DNA records in a non-CODIS local DNA database will not be searched against the DNA records contributed by over 200 submitting forensic DNA laboratories that participate in the National DNA Index System.

Conclusion

The SAFER Act of 2013 drew attention to the volumes of untested sexual assault evidence kits nationwide and has resulted in a huge influx of these kits to forensic laboratories for analysis. This projected backlog has forced forensic laboratories to evaluate their options to provide a more timely public service. Since many forensic laboratories will not have the resources to recruit and train additional personnel to handle the increased backlog, they must reexamine how these cases will be analyzed.

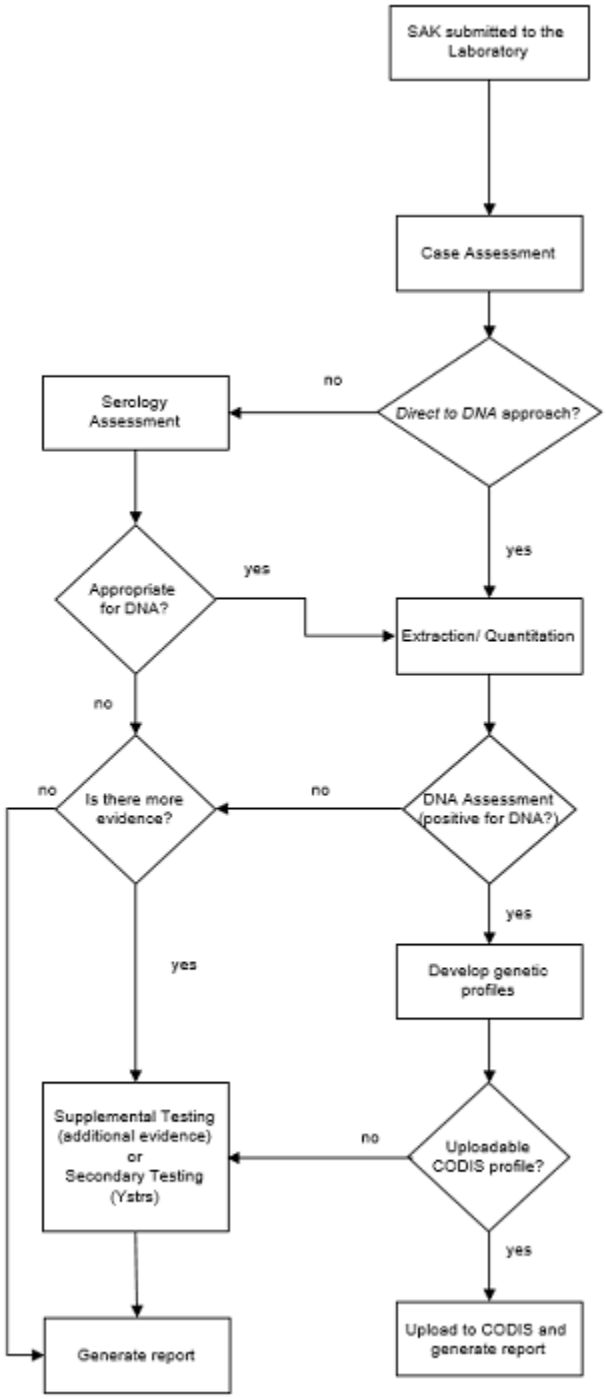
[17] A checklist for law enforcement agencies that are considering outsourcing is provided in Appendix 3 to ensure that the DNA records generated by the vendor laboratory are eligible for CODIS.

In order for laboratories to increase efficiency long term and decrease overall turn-around time, they must examine alternative approaches and embrace new technologies. Robotic extraction, coupled with the *Direct to DNA* approach, has been implemented successfully in multiple laboratories to analyze cases more efficiently. Use of an internally validated Y chromosome quantification threshold not only utilizes a more sensitive technique than sperm searching for the detection of male DNA, but also may alleviate further analysis and typing of low-level samples that would otherwise yield no useful typing information. Additional strategies such as evidence submission policies to limit testing to mutually prioritized items should also be considered.

Regardless of how many of the foregoing recommendations are implemented by a laboratory, the goal is to assist a laboratory to achieve a more efficient processing of sexual assault evidence kits and ultimately reduce the turn around time for the analysis of the kits.

Appendix 1

High Throughput Process Flow for Sexual Assault Evidence Kits



Appendix 2

Persistence of Body Fluids/DNA Articles

BODY FLUID	REFERENCES
Saliva (skin)	M.M. Stark, *Clinical Forensic Medicine: A Physician's Guide* (2011). J. Kenna, et al., *The Recovery and Persistence of Salivary DNA on Human Skin*, J. Forensic Sci. Vol. 56, No. 1 (2011).
Semen (vaginal)	Macaluso et al., *Prostate-specific antigen in vaginal fluid as a biologic marker of condoms failure*. Contraception 59:195-201 (1999). K. A. Mayntz-Press, L. Sims, A. Hall, J. Ballantyne, *Y-STR Profiling in Extended Interval (≥ 3 days) Postcoital Cervicovaginal Samples*, J. Forensic Sci. 53: 342-348 (2008).
Sperm/Spermatozoa (oral cavity and oral swabs)	W.F. Enos, J.C. Beyer, *Spermatozoa in the Anal Canal and Rectum and in the Oral Cavity of Female Rape Victims*, J. Forensic Sci. Vol. 23: 231-233 (1978). G. M. Willott, J.E. Allard, *Spermatozoa – Their Persistence After Sexual Intercourse*, Forensic Science International, 19:135—154 (1982).
Sperm/Spermatozoa (anal canal/rectal swabs)	W.F. Enos, J.C. Beyer, *Spermatozoa in the Anal Canal and Rectum and in the Oral Cavity of Female Rape Victims*, J. Forensic Sci. Vol. 23: 231-233 (1978). G. M. Willott, J.E. Allard, *Spermatozoa – Their Persistence After Sexual Intercourse*, Forensic Science International, 19:135—154 (1982).
Sperm/Spermatozoa (cervix)	E. M. Silverman, A.G. Silverman, *Persistence of Spermatozoa in the Lower Tracts of Women*, Journal of American Medical Association, Vol. 240, No. 17 (1975). J.P. Allery, N. Telmon, R. Mieusset, A. Blanc, D. Rouge, *Cytological detection of spermatozoa: comparison of three staining methods*, J. Forensic Sci, 46: 349-351 (2001). E. K. Hanson and J. Ballantyne, *A Y-STR Specific DNA Enhancement Strategy to Aid the Analysis of Late Reported (> 6 days) Sexual Assault Cases*. *Med Sci Law* 54 (4) 209-218. doi: 10.1177/0025802413519761 (2014). E. K. Hanson, J. Ballantyne, *Enhanced DNA Profiling of the Semen Donor in Late Reported Sexual Assaults: Use of Y-Chromosome-Targeted Pre-amplification and Next Generation Y-STR Amplification Systems*. Methods Mol. Biol. 1420:185-200 (2016); doi: 10.1007/978-1-4939-3597-0_15 (in process).
Sperm/Spermatozoa (external and internal vaginal swabs)	G. M. Willott, J.E. Allard, *Spermatozoa – Their Persistence After Sexual Intercourse*, Forensic Science International, 19:135—154 (1982). B. B. Hellerud et al., *Semen detection: A retrospective overview from 2010*, Forensic Scence International Series 3: 391-392 (2011).
Sperm/Spermatozoa (posterior fornix)	E. Hanson and J. Ballantyne, *A Y-STR Specific DNA Enhancement Strategy to Aid the Analysis of Late Reported (> 6 days) Sexual Assault Cases*. *Med Sci Law* 54 (4) 209-218. doi: 10.1177/0025802413519761 (2014). E. K. Hanson, J. Ballantyne, *Enhanced DNA Profiling of the Semen Donor in Late Reported Sexual Assaults: Use of Y-Chromosome-Targeted Pre-amplification and Next Generation Y-STR Amplification Systems*. Methods Mol. Biol. 1420:185-200 (2016); doi: 10.1007/978-1-4939-3597-0_15 (in process).
Sperm/Spermatozoa (clothing)	E. Kafarowski, A.M. Lyon, M.M., Sloan, *The retention and transfer of spermatozoa in clothing by machine washing*, Can. Soc. Forens. Sci. J. 29: 7-11 (1996).

Appendix 3: Outsourcing Sexual Assault Evidence Kits

Laboratories and law enforcement agencies may contract out (outsource) the analyses of DNA samples in accordance with State and Federal law. To ensure the eligibility of the resulting DNA records for CODIS and the National DNA Index System (NDIS), these agencies must comply with the outsourcing requirements specified in the Federal Bureau of Investigation (FBI) Director's *Quality Assurance Standards for Forensic DNA Testing and Databasing Laboratories*. For law enforcement agencies seeking to outsource sexual assault evidence kit (casework) samples, the technical specifications of the outsourcing agreement must have the prior approval of the technical leader of the NDIS participating laboratory taking ownership of and entering that DNA data into CODIS.

Law enforcement agencies intending to outsource sexual assault evidence kits must obtain the PRIOR APPROVAL for the technical specifications of the outsourcing agreement from the technical leader of the NDIS participating laboratory taking ownership of the DNA data in order for that data to be eligible for CODIS.

Law enforcement agencies seeking to outsource must ensure that the vendor laboratory follows the FBI's *Quality Assurance Standards* and is accredited. The *Quality Assurance Standards* also requires the completion of an on-site visit of the vendor laboratory prior to the beginning of the outsourced analyses and a technical review of the outsourced DNA records by the NDIS participating laboratory that will be taking ownership of those records. Prior to the submission of DNA samples to the vendor laboratory, agencies must ensure compliance with the following outsourcing checklist of requirements:

Outsourcing Checklist

Requirement	Law Enforcement Agency	NDIS Participating Laboratory	Vendor Laboratory
Vendor Laboratory accredited by approved accrediting agency	✓	✓	✓
Vendor Laboratory complies with FBI Director's *Quality Assurance Standards*	✓	✓	✓
Vendor Laboratory required to use same technology, platform and typing amplification test kit as that used by NDIS Laboratory taking ownership of DNA records	✓	✓	✓
Technical Leader of NDIS Laboratory has approved the technical specifications of the outsourcing agreement prior to its award	✓	✓	
On-site visit of the Vendor Laboratory prior to any outsourced DNA analyses	✓	✓	✓

Source: FBI Director's Quality Assurance Standards for Forensic DNA Testing Laboratories available at http://www.swgdam.org/publications.

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Purps, J., Geppert, M., Nagy, M., Roewer, L., *Validation of a combined autosomal/Y-chromosomal STR approach for analyzing typical biological stains in sexual-assault cases*. Forensic Sci Int Genet. 19:238-42 (2015).

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