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Improving Laboratory Efficiency: A Direct-to-DNA Approach for Testing Sexual Assault Kits

Comparing Biological Fluid Screening to a Direct-to-DNA Approach

Biological fluid screening has been a hallmark in criminal investigations since it started in forensics, well before DNA analysis became a possibility. However, a direct correlation does not always exist between (1) the presence or absence of a biological fluid and (2) the ability to generate a DNA profile from the same source of biological evidence.

Acid phosphatase (AP), the main component in an accepted screening approach used to identify semen, degrades over time—leading to an unreliable screening tool for cold case sexual assaults.[1] Following AP screening, especially for negative or inconclusive results, the best practice is to conduct additional microscopic examination of the evidence to determine the presence of sperm cells; this procedure helps to verify the AP results. Laboratories spend significant amounts of time and resources and also consume evidentiary sample screening cases for biological fluids using methods that have not advanced as quickly as DNA testing methods.

Today's DNA technology is highly sensitive, lending to the possibility of obtaining DNA profiles suitable for comparison and eligible for the Combined DNA Index System (CODIS) from digital penetration cases, vasectomized males, and degraded/aged samples. Additionally, this technology enables fully automated laboratory processing of sexual assault kits (SAKs)—thereby preserving sample, reducing human error, and decreasing laboratory testing time.

With these advantages in mind, the Sexual Assault Forensic Evidence Reporting (SAFER) working group[i] recommends a direct-to-DNA approach for testing SAKs; the SAFER working group also recommends performing serology testing on an as-needed basis.[2] Agencies facing legislative changes in the SAK submission and/or testing parameters need an efficient method for identifying and processing the evidentiary samples with the most potential for a probative, CODIS-eligible profile; a direct-to-DNA approach can address these needs.

This brief highlights the benefits of a direct-to-DNA approach—not only for SAK testing, but also for laboratories, prosecution, and law enforcement.

Improving DNA Analysis for Sexual Assault Cases

A direct-to-DNA approach utilizes the quantification step in the DNA testing process to detect the level of male DNA among female DNA collected and extracted from vaginal swabs. This approach is ideal for sexual assault evidence because 91% of rape and sexual assault victims are female and 95% of offenders are male, and samples typically consist of a mixture of both male and female cells/DNA.[3]

DNA extraction removes DNA from a swab or another substrate (e.g., underwear). Afterward, the amounts of overall human DNA and male DNA in the sample are estimated as part of the quantification step. During this step, a sample is also evaluated to determine if it is likely to generate a CODIS-eligible DNA profile rather than relying on the results of biological fluids analysis. Evaluation at this step is based not only on the amount of male DNA present but also the ratio of total human DNA to male DNA, which further clarifies the ability to obtain a CODIS-eligible DNA profile.

Recognizing Positive Outcomes: A Direct-to-DNA Approach

A direct-to-DNA approach has been reliably used in various forms on most major backlog reduction efforts involving SAK testing. By using procedure variations, laboratories can select methods that best fit their size, structure, and jurisdictional needs when evaluating how to implement a direct-to-DNA approach for SAK processing. A direct-to-DNA approach increases laboratory efficiency by removing time-consuming steps such as sampling items independently for serology and microscopic examination, especially for negative samples; reducing labor and resource consumption; increasing the number of DNA profiles entered into CODIS; and giving the laboratory the ability to gain additional efficiency through the use of full automation.

[i]This group was created in response to the Sexual Assault Forensic Evidence Reporting Act of 2013; this act advises developing best practices related to collecting and processing DNA evidence in sexual assault cases.[2]

Evaluating Procedural Considerations

Laboratories have several direct-to-DNA approaches to consider for workflow implementation. These approaches include utilizing a vendor screening procedure, utilizing the laboratory's standard differential extraction and quantification methods, and using a nondifferential extraction and then determining which sample(s) to re-extract using a differential process.[4–6]

A laboratory should consider multiple aspects of the workflow prior to deciding which direct-to-DNA approach to adopt. The following list provides several considerations:

- **Establish detection limits for sample selection.** Samples will be selected based on the amount of male DNA present. Minimum detection limits to obtain a DNA profile for autosomal and male DNA must be established.
- **Define parameters for determining which short tandem repeat (STR) method to use.** Human-to-male-DNA ratios must also be established to determine when autosomal STR testing or Y-STR testing would be the better method. Reviewing the quantification validation can help determine if additional studies are necessary to establish these limits for the laboratory's chosen amplification kit.
- **Create a strategy for case and sample processing.** Once detection limits have been established, laboratories can generate procedures to triage cases and select the type and number of samples that will proceed to DNA testing. The laboratory must determine how many samples per SAK to submit for DNA testing and how many swabs to process if multiple swabs have been taken for an orifice; additionally, the laboratory will need to decide if items such as underwear and pubic hair combings will be included for DNA testing. Laboratories may want to evaluate their sample selection process to determine the baseline data obtained from consuming one entire swab, taking small cuttings from multiple swabs collected per orifice, or other variations. Through these studies, laboratories may also be able to determine ratios for when it is possible to infer that the detected male DNA most likely came from sperm cells.[7]

Inferring a DNA Profile Originated from Sperm Cells

The differential extraction procedure was developed to separate sperm cells from non-sperm cells; this procedure is based on a sperm cell's physical properties and the chemical modifications that are required to remove DNA. With these principles in mind, Anderson, et al., describe a process for identifying criteria under which a DNA profile can be reliably inferred as coming from spermatozoa.[7] As predicted, their results indicate that—under varying conditions—male DNA from spermatozoa is successfully enriched in the fraction that contains the sperm pellet over other biological fluids, such as blood and saliva.

Performing Serological Testing After a Direct-to-DNA Approach

Certain circumstances may warrant biological fluid screening, even after obtaining a DNA profile suitable for comparison. Laboratories should consider how to preserve sample for possible future testing—including biological fluid screening, Y-STR analysis, and other advanced methods. Retaining sample by not consuming all the substrate or by spotting the "sperm fraction pellet" during differential extraction can create a way to verify biological fluid, if necessary.

Encouraging Interagency Communication

Engage law enforcement and prosecutor offices early to make them aware of any planned changes in triaging and screening cases; communicating this information helps them to determine how those changes may impact their requests for testing or their need for investigation and prosecution. Being prepared to implement the new approach is a big change for all parties involved. Listening to law enforcement and prosecutors' feedback is important to understanding how the change will affect their daily work. Having a method to provide biological fluid testing, if requested, can help with the adjustment. For these situations, the laboratory should create a streamlined method for submitting requests. Additionally, following up after implementing a direct-to-DNA approach will allow all stakeholders to express any concerns and define a path for the future.

Spotting a Sperm Pellet

The sperm fraction can be spotted onto a prepared microscope slide if the presence of spermatozoa needs to be confirmed. Following removal of the epithelial supernatant, resuspend the sperm pellet (e.g., in sterile water or a Tris/EDTA buffer), remove an aliquot of the pellet suspension, and spot on a slide according to laboratory procedures. Follow standard procedures for staining and microscopic examination.

Implementing a Direct-to-DNA Approach

Six laboratories in the Florida Department of Law Enforcement (FDLE) laboratory system implemented a direct-to-DNA approach after new legislation became effective in July 2016. The legislation, Florida Statute 943.326, requires the submission and testing of all SAKs from a reporting victim.[8] A direct-to-DNA approach helped to prepare for the increase in SAK submissions. The laboratory obtained robotic instrumentation to perform an automated differential extraction procedure and conducted testing of procedural modifications to triage cases for sample selection. This testing also helped to determine detection limits to use in a direct-to-DNA approach.

The laboratory uses a direct-to-DNA approach on sexual assault cases involving a female victim and male perpetrator(s). An entire swab from each orifice where semen may have been deposited is sampled and subjected to a differential extraction process; an entire swab is sampled for all non-orifice body swabs. Based on the source (e.g., possible semen, saliva, or touch DNA) of potentially foreign DNA, extraction proceeds either differentially or nondifferentially. Underwear collected during the forensic examination will be sampled and will undergo a differential extraction. For samples that have a sufficient amount of male DNA, the specific circumstances of the case (e.g., multiple perpetrators, loss of consciousness, prior consensual encounter) will dictate the number of samples that move forward for DNA amplification. A case with a single perpetrator and no prior consensual activity will have fewer samples amplified than a case with prior consensual activity or multiple perpetrators.

To examine the impact of a direct-to-DNA approach, FDLE's Jacksonville laboratory selected a batch of 51 SAKs (Batch A) that were processed using a biological fluid screening approach and compared it to a batch of 48 SAKs (Batch B) that were processed using a direct-to-DNA approach. Approximately half of the samples in each batch did not proceed to DNA testing. In other words, not microscopically examining negative cases led to immediate time and resource savings in roughly half of these direct-to-DNA cases. Of the remaining samples that proceeded with DNA testing in Batch A, 32 of 51 (62%) biological fluid–screened cases contained samples that were uploaded to CODIS compared to 41 of 48 (85%) direct-to-DNA cases in Batch B. These numbers demonstrate that a direct-to-DNA approach is just as good, if not better, at screening samples suitable for DNA testing compared to biological fluid screening.

All six FDLE laboratories implemented a direct-to-DNA approach from July 2016 through September 2018. During this time, the laboratories received 6,209 SAKs and tested 5,546 SAKs.[9] Stakeholders understood the resource limitations the laboratories faced and the probative importance of producing DNA profiles from these SAKs; therefore, stakeholders chose a direct-to-DNA approach over spending time and resources biologically screening cases up front. During this timeframe, the Jacksonville laboratory had only a single case in which spermatozoa detection was requested following DNA testing. Evidence was preserved in this case and screening was performed. A direct-to-DNA approach has been a significant efficiency gain for the FDLE laboratory system, allowing it to have 99.9% compliance with the mandated 120-day turnaround time.

Advantages of a Direct-to-DNA Approach

Capability	Advantage of Capability
Predicts DNA testing success	Meets or exceeds traditional biological screening tests.
Enhances DNA testing efficiency while conserving resources	Provides a significant gain in efficiency, allowing laboratories to meet testing time mandates.
Conducts a sperm verification test	Can preserve the ability to verify the presence of sperm, if requested.

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