Reducing manual steps and optimizing automation will be essential as laboratories look for sustainable methods to enhance the efficiency of processing sexual assault kits (SAKs). Many forensic laboratories screen SAKs for biological fluid, such as semen; this process can take approximately 4–7 hours per kit. Following biological fluid screening, the samples deemed probative for possible DNA are selected for differential extraction—a process that separates sperm cells from non-sperm cells. The trained capacity and number of hours needed to screen thousands of kits, let alone proceed with DNA testing, can cripple crime laboratories. Thus, laboratories need alternative approaches to reduce bottlenecks in the screening process.

Male DNA Screening

Male DNA screening—also known as Y-Marker screening or Direct-to-DNA—has been used in large outsourcing projects, such as the testing of SAKs in New York City, Los Angeles, Houston, and Detroit. However, this approach has not been widely adopted throughout public and government crime laboratories.

Male DNA screening shifts the emphasis from screening cases for biological fluid to screening cases with quantification, a step already used in DNA processing to determine how much DNA a sample contains. Because modern commercial quantification kits determine how much (1) human DNA (i.e., both male and female DNA) and (2) male DNA a sample contains, quantification can be used in the screening process and can take less than 1 hour per kit.

The screening portion shifts to a step that already exists in DNA processing; therefore, male DNA screening can be automated for high-throughput testing. Male DNA screening does not detect seminal fluid or semen. However, screening for male DNA will help detect possible probative samples from cases involving digital penetration or other touching, no ejaculation, or azoospermic males who currently screen negative in biological fluid screening. In one study, male DNA screening proved more effective than biological fluid screening at detecting male DNA from vaginal swabs, external genitalia, and dried secretions. For cases that may have been improperly stored or aged, male DNA screening may be a better predictor of the success of obtaining a DNA profile where enzymes that detect biological fluids may have degraded.

Methods of Male DNA Screening

Male DNA screening is commonly applied in two ways—the difference between the approaches is when the screening step occurs in relation to the differential extraction.

Approach A: Screen Before Differential Extraction

With this approach, a small cutting is taken from a sample. A fast and crude DNA extraction is performed, then the sample proceeds to quantification with male DNA screening. This approach quickly identifies any male-DNA-negative samples, allowing a scientist to proceed with DNA analysis by returning to the evidence and taking another sampling of male-DNA-positive samples.

Approach B: Screen After Differential Extraction

As part of this approach, a larger cutting is taken for DNA testing. A differential extraction is performed, then the sample proceeds to quantification with male DNA screening. This approach identifies any male-DNA-negative samples, allowing a scientist to proceed with DNA testing on the remaining extract for male-DNA-positive samples.

Workflow Considerations: Choosing the Better Approach

<table>
<thead>
<tr>
<th>Approach A</th>
<th>Approach B</th>
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<tbody>
<tr>
<td>Uses less sample</td>
<td>Is more labor intensive on the front-end</td>
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<tr>
<td>Is less labor intensive upfront</td>
<td>Is more efficient in workflows using automation of differential extractions or sperm preferential extractions</td>
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<table>
<thead>
<tr>
<th>Approach A</th>
<th>Approach B</th>
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<tbody>
<tr>
<td>Requires male-DNA-positive evidence to be sampled and extracted a second time</td>
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</table>
Male DNA Screening Is Not Y-STR Testing

Male DNA screening occurs during the quantification stage of DNA testing. Estimates of the amount of total human DNA and male DNA present in the sample occur during quantification. The estimate of male DNA and the ratio of human DNA to male DNA are used to guide further DNA testing, such as short tandem repeat (STR) and/or Y-STR testing.

Y-STR testing occurs during the amplification stage of DNA testing and follows quantification. Y-STR testing targets short tandem repeat loci found on the male Y-chromosome. Y-STR testing may be useful in the following situations:

- To analyze a sample when a large amount of female DNA exists in the presence of a small amount of male DNA
- To clarify the number of males in a sample
- To provide clarity for inconclusive STR results
- To aid in the power of exclusion.

Male DNA Reporting

Although consistent reporting guidelines do not exist for male DNA screening established in the United States, some trends have been identified. Generally, results will fall into one of five categories:

1. Positive for male DNA (i.e., male DNA detected and suitable for DNA testing)
2. Positive for male DNA but in the presence of a high ratio of total human DNA (i.e., male DNA detected but generally unsuitable for STR testing, though it may be suitable for Y-STR testing)
3. Negative for male DNA (i.e., no male DNA detected above the detection threshold)
4. No results (i.e., no DNA present above the detection threshold)
5. Inconclusive (i.e., based on the assay it is not possible to confirm reliably the presence of male DNA).

Summarizing Case Studies

Case metrics from New York, Los Angeles, Detroit, and Houston can be useful in decision making. Among the 4 jurisdictions, 7,811 kits were analyzed for metrics. Of these kits, eligibility for query against the Combined DNA Index System (CODIS) was 35%–65% and CODIS hit rates were 13%–29%. The New York project has been completed for more than a decade, and so now there is more information on post-CODIS hit follow-up—especially from Manhattan. Of the cases in which an assailant was identified via DNA, the case did not proceed to indictment when the statute of limitations was expired or the victim was missing, recanted, or did not want to proceed. As such, the indictment rates are currently fewer than 2%. Shifting semen confirmation to the indictment stage would significantly decrease the time and effort involved in laboratory processing.

Unless semen detection is required for a particular legal charge, a DNA profile may be the only evidence needed to proceed with indictment.

Moving Forward

Some multi-disciplinary working groups that have been formed through recent grant initiatives have determined that identifying biological fluid could be essential to the case. However, this does not mean utilizing biological screening as the means to identify probative DNA is necessary. Laboratories that use Y-Marker screening often have protocols in place to perform serological tests for identifying biological fluid if specifically requested.

As agencies have moved into other phases of their backlog projects, multi-disciplinary teams have determined that biological fluid screening could be eliminated. As a result, a male DNA screening approach is being adopted throughout agencies, such as the Michigan State Police, for all sexual assault kits.

Conclusion

Male DNA screening will not identify biological fluid or tissue type; however, this approach has many benefits that may not be realized with a biological fluid screening approach. These benefits include the following:

- Automate high-throughput processing
- Reduce cost and time to screen a SAK
- Increase identification of probative samples submitted for DNA
- Aid in determining success of obtaining a DNA profile.

Effective education, training, and communication among law enforcement officials, prosecutors, and forensic scientists can determine optimized approaches to address legislative requirements and utilize modern forensic technology.
References:


Author:

Amy Jeanguenat, MFS, has spent her career working in the private forensic industry supporting efforts worldwide to prevent and eliminate DNA backlogs. Mrs. Jeanguenat helped manage the successful completion of sexual assault kit outsourcing projects from Houston, Texas, and Detroit, Michigan. Currently Mrs. Jeanguenat works as the principal consultant at Mindgen, LLC.

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