Y9 A Comparison of Semi-Automated and Manual Differential Separation Methods for Mock Sexual Assault Swabs

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Learning Overview: After attending this presentation, attendees will be familiar with the SpermX™ differential separation method and understand how this novel, manual procedure compares to the semi-automated differential separation protocol of the Federal Bureau of Investigation (FBI) Laboratory Unit when used to extract male and female DNA from mock sexual assault samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating how new developments in DNA technology can be evaluated by comparing alternative procedures with validated protocols.

In the event of a sexual assault, separation of suspect from victim DNA is possible through differential extraction if the evidentiary mixture consists of female epithelial cells and male spermatozoa. The basis of differential extraction is the differing properties of epithelial and sperm cell membranes, specifically the comparative sturdiness of the proteins making up the sperm head containing the male DNA. Effective retrieval and separation of male and female DNA fractions is important in generating high-quality Short Tandem Repeat (STR) profiles that can be utilized in identifying suspect(s) in a sexual assault case.

Differential extraction has been conventionally performed using Proteinase K (PK) to lyse epithelial cells, then Dithiothreitol (DTT) to lyse sperm cells after the fractions were separated through centrifugation. The protocol for differential separation currently used by the FBI Laboratory is a semi-automated variation of this method, with the use of the QIAcube® for separation of female and male cells, and the EZ1™ Advanced XL for DNA purification. SpermX™ is a manual differential separation method that uses a novel nanofiber matrix to separate sperm from epithelial cells. As a cellular mixture is washed through the SpermX™ device, epithelial DNA lysate will flow through while sperm cells are trapped within the matrix. Sperm DNA is released using a sperm digest buffer and extracted with the EZ1™ Advanced XL. Comparison of the FBI and SpermX™ methods through mock sexual assault sample extraction may indicate an alternative differential method that can be used if the current automation is unavailable.

A solution of female epithelial cells was prepared by washing neat saliva and resuspending the resulting pellet in Tris-EDTA (TE) buffer. Human semen from five different male donors, acquired from a commercial source, was diluted 1:20, and equal volumes of epithelial solution and semen dilution were evenly distributed onto cotton swabs. Mock sexual assault samples were quantified with Quantifiler® Trio. Following quantification, samples were amplified with GlobalFiler® Polymerase Chain Reaction (PCR) Amplification Kit and genotyped using the 3500xL Genetic Analyzer. Short Tandem Repeat (STR) profiles were analyzed using GeneMapper® IDX v1.6.

Comparisons between the FBI and SpermX™ differential protocols were made from the samples' DNA quantity, profile quality, and degradation level for each of the male and female differential fractions. The female fractions of both methods were similar in containing a mixture of male and female DNA. They also both yielded high-quality, non-degraded DNA as indicated by their degradation indices, which were 1.04 and 1.00 for the FBI method and SpermX™, respectively. There was no significant difference between the female fraction DNA quantities, which ranged from 0.14 to 5.32ng/uL for the FBI method and 1.57 to 6.20ng/uL for SpermX™ ($p>0.05$). Therefore, the FBI and SpermX™ differential methods are comparable when extracting female DNA from a mock sexual assault swab.

When observing the male fraction, the FBI and SpermX™ methods were similar in separation success and DNA quality. Both methods yielded clean male fractions, showing success in differential separation. The degradation indices of the male fractions were 0.79 for the FBI method and 0.78 for SpermTrap™, which indicates high-quality, non-degraded DNA. Unlike the female fractions, there was a significant difference in male DNA quantity. The DNA quantity extracted by the FBI method ranged from 0.14 to 5.32ng/uL, which was significantly larger than the quantity of male DNA extracted by the SpermX™ method, which ranged from 0.04 to 2.02ng/uL ($p<0.05$). Although both differential extraction methods produced a well-separated, high-quality male fraction, the FBI method was able to extract a higher quantity of male DNA from mock sexual assault swabs.

The DNA analysis of mock sexual assault swabs enabled the comparison of two differential separation methods, showing the FBI and SpermX™ methods are of comparable quantity and quality.

Differential Extraction, SpermX™, STR Profile

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