VERSA 1100

OVERVIEW OF THE ORIGINAL AND CURRENT DIFFERENTIAL DIGESTION USING SELECTIVE DEGRADATION PROCESS ON VERSA1100

Introduction

Forensic laboratories worldwide are under continuous pressure to process DNA evidence from sexual assault investigations, often surpassing their current throughput capabilities. Sexual assault samples, collected from bodily fluids, tissues, clothing, and other materials, typically contain a mix of cells from both the alleged perpetrator and the victim. Additionally, DNA in these samples may be degraded or present in low concentrations, complicating the process of obtaining a clear DNA profile for individualization.

The predominant method for analyzing sexual assault samples involves separating sperm cells from all other cell types present. Most current protocols for isolating sperm cells rely on the differential chemical lysis of epithelial cells from the victim, followed by manual centrifugation and washing steps. This process separates DNA from non-sperm cells, mainly of female origin, from any sperm cells. However, this technique is lengthy, labor-intensive, and challenging to automate. In response, alternative methods like automation have been developed to assist in processing sexual assault samples.

This App Note highlights Aurora VERSA1100 aimed at improving or complementing Differential Digestion extraction (DD)—the process that separates spermatozoa from non-sperm cells in forensic mixtures. While not exhaustive, this paper focuses on key developments that leverage unique chemistries and physics at the microscale level.

Analysis and Backlog of Sexual Assault Evidence

Forensic laboratories process hundreds of thousands of biological evidence samples annually, with approximately three-quarters requiring DNA analysis. This involves isolating DNA from samples and removing inhibitors that affect DNA amplification. Sexual assault evidence samples often contain cellular mixtures from multiple contributors, necessitating the separation of sperm and non-sperm cells before DNA profiling.

The manual protocols required to process Sexual Assault Evidence Collection Kits (SAECKs) contribute to significant backlogs in DNA analysis. Despite federal funding and increased staffing, the demand for forensic DNA casework has outpaced laboratory throughput. This backlog profoundly impacts society, as victims may wait months or years for DNA evidence to further investigations and identify attackers.

Recent initiatives by the United States government aim to address this issue by increasing funding and prioritizing the rapid processing of sexual assault samples.

In this application note, we will explore the latest updates in differential extraction process, highlighting how these advancements can improve the efficiency and effectiveness of processing sexual assault evidence in forensic laboratories.

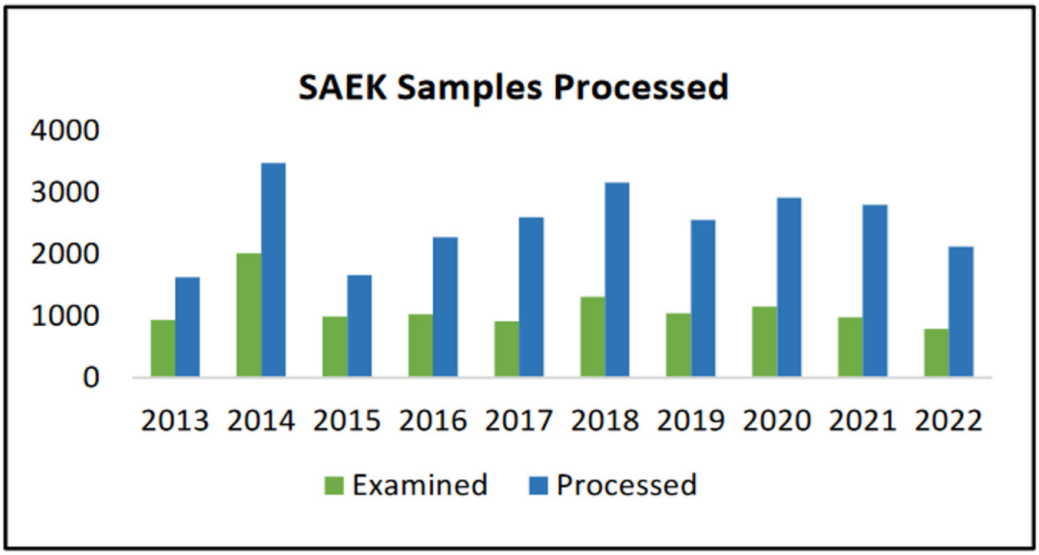


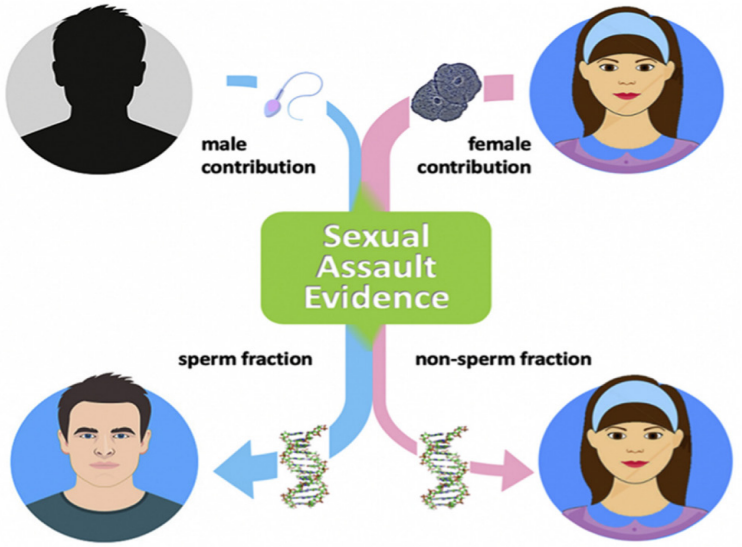
Fig1: Total number of SAEK samples examined and processed (extracted) annually the Oakland Police Department Criminalistics Laboratory

The Differential Extraction Process

Conventional Differential Extraction

Typically, sexual assault samples undergo preferential lysis to break open non-sperm cells and release their genetic material while leaving sperm cells intact. This initial digestion of epithelial cells is performed using proteinase K (Pro-K) and sodium dodecyl sulfate (SDS), followed by centrifugation and washing to recover the sperm cell pellet. The sperm cells are then lysed using surfactant, Pro-K, and dithiothreitol (DTT), resulting in a purified sperm fraction ideally containing only DNA from the assailant or consensual partner.

1. Male Fraction: A solution of male DNA that is completely free of any DNA or cellular material from the victim. This fraction, often called the sperm or male fraction, should ideally be single-source when only one male contributor is present.
2. Female Fraction: A solution containing DNA from the victim and any non-sperm cells from the male donor.



Once male DNA is isolated, forensic analysts proceed with DNA quantification, amplification, and separation using qPCR, PCR, and capillary electrophoresis, respectively. Conventional DE methods typically yield high-quality DNA profiles, providing valuable evidence for criminal investigations.

Shortcomings of Conventional Differential Extraction

The process involves multiple centrifugation and washing steps, each requiring five minutes or longer and repeated up to five times to achieve a clean male fraction. The number of wash repetitions varies between laboratories and cannot be predetermined using traditional microscopy. Although effective at isolating sperm cells, this process is time-consuming, potentially taking several hours per sample, and requires skilled forensic analysts.

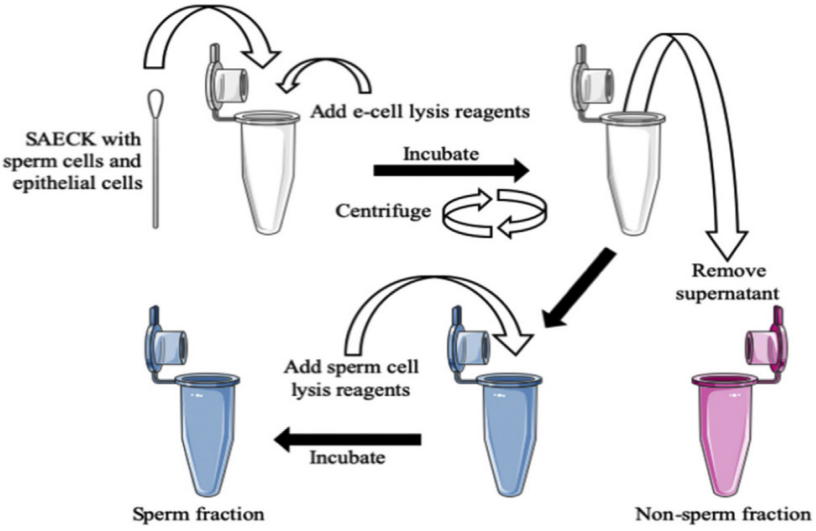


Fig. 2. Schematic of a traditional differential extraction protocol. A cutting of the sample containing sperm cells and epithelial cells is combined with epithelial cell lysis reagents, liberating non-sperm DNA. Using centrifugation, sperm cells are pelleted to the bottom of the tube, and the supernatant is removed as the non-sperm fraction. Sperm cell lysis reagents are added to the pellet of sperm cells and incubated, breaking them open and

The quality of separation can vary significantly between labs and individual analysts, depending on the age and condition of the evidence sample. Variable incubation times further contribute to inconsistencies. Commercial kits and reagents are available, but they still involve multiple manual washing steps and may require additional DNA concentration post-isolation.

The time constraints associated with sample preparation and downstream processing, such as cellular lysis, DNA quantification, DNA amplification, and DNA separation, remain constant regardless of the technique. The manual washing and centrifugation steps are particularly problematic due to difficulty in automation and contamination risks with each treatment. Therefore, research efforts are focused on developing alternative methods to capture sperm cells more effectively, reducing analyst time and contamination potential.

Alternative approaches to conventional method

Selective Degradation of Female DNA

While traditional methods for obtaining single-source male STR profiles from sexual assault samples rely primarily on the physical separation of sperm cells from non-sperm material, a nuclease-only approach has been developed. This method involves a single centrifugation step to concentrate sperm cells, followed by the use of protease and sodium dodecyl sulfate (SDS), for epithelial cell lysis. Subsequently, DNase I enzyme is used to completely remove all female DNA from the sperm pellet before lysing the sperm cells. This protocol demonstrated that the nuclease method can generate male STR profiles of equal or higher quality than traditional methods.

During differential extraction, the epithelial cells are first selectively lysed through the addition of SDS and proteinase K. Sperm cells are generally resistant to lysis by these chemicals. Once the epithelial cells have been lysed, centrifugation is used to selectively pellet any intact sperm cells. The supernatant-containing DNA from male and female epithelial cells can then be removed to a separate tube. The isolated sperm cells are then lysed by the addition of SDS, proteinase K, and dithiothreitol (DTT). DTT reduces the disulfide bonds present in the sperm nuclear membranes, releasing sperm cell DNA. The DNA in the separate epithelial cell fraction and the sperm cell fraction can then be isolated by organic or nonorganic methods. This extraction method can simplify the interpretation of DNA profiling results by increasing the likelihood of obtaining a clear male DNA profile from the sperm fraction. Since the epithelial fraction contains both male and female DNA, this may still present as a DNA mixture. It should be emphasized that this is a pre-PCR approach that physically isolates male cells for autosomal STR analysis, which offers a superior power of discrimination.

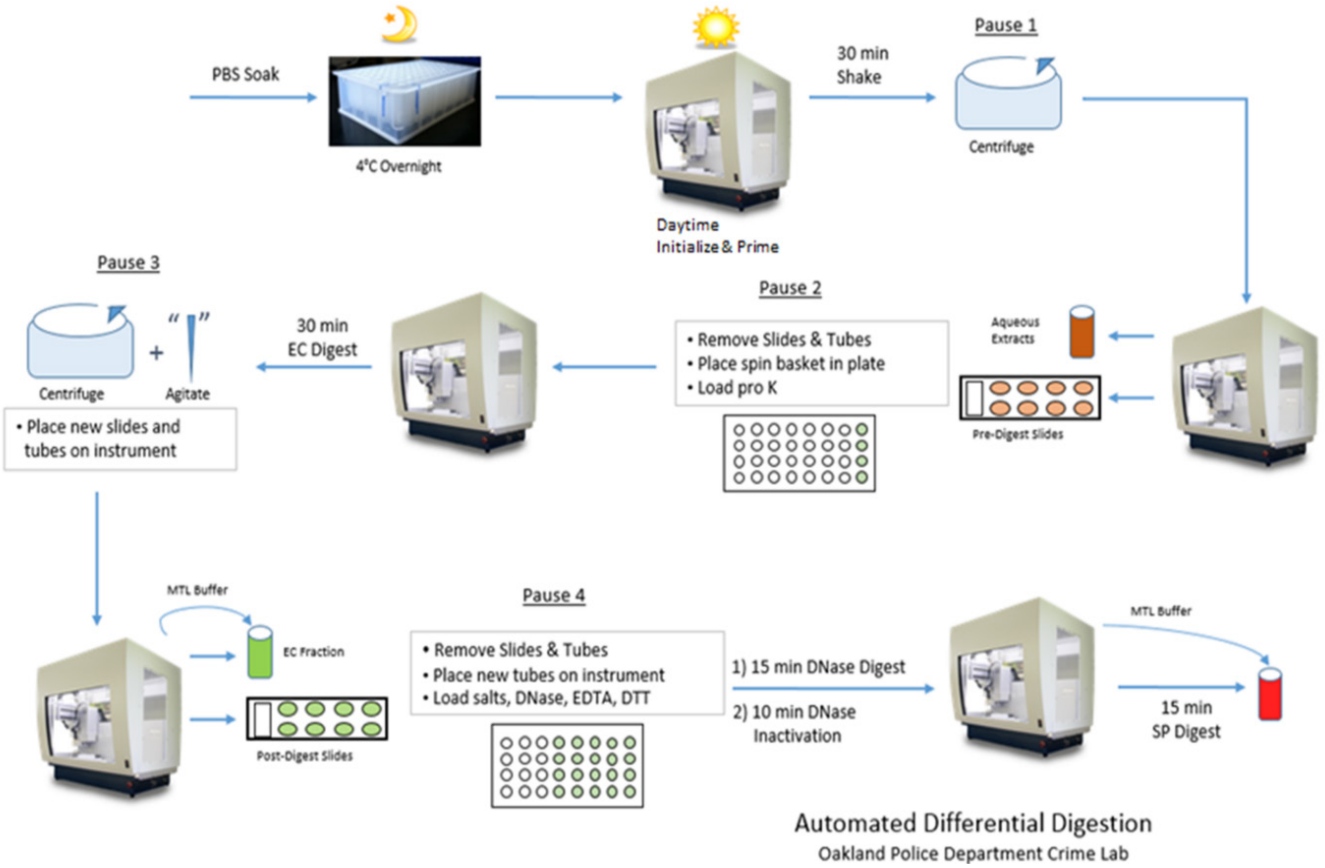


Fig3: Automated Differential Digestion protocol automated on Validated VERSA1100 automated liquid handler workstation by Oakland Police Department

To automate this approach, selective degradation of female DNA was combined with the VERSA 1100 liquid handling station (Aurora Biomed). The automated method provided similar DNA yields to the manual selective degradation approach, with excellent reproducibility across multiple replicates.

In a study published by OPD, the VERSA workstation demonstrated that automated selective degradation could yield results comparable to those obtained through traditional protocols. Remarkably, this was achieved with a six-fold increase in speed and without the necessity for analyst intervention. Over time, the protocol has been refined; now, samples are soaked in PBS for only 30 minutes before initiation and priming. Further optimizations have been made, allowing for the customization of automated protocols with or without slide preparation on deck, depending on specific requirements. This advancement highlights the VERSA workstation's capability to streamline laboratory workflows while maintaining high standards of accuracy and efficiency.

Fig4: Automated Differential Digestion protocol automated on Validated VERSA1100 automated liquid handler workstation by Oakland Police Department with and without slide preparation.

Conclusion

While the effectiveness of the selective degradation method (whether manual or robotic) makes it a valuable addition to traditional DNA analysis, it has not yet replaced the conventional process in most forensic laboratories. The potential for automating Differential Digestion Extraction through selective degradation could easily be expanded to accommodate multiple plate automation for high-throughput protocols, significantly enhancing the efficiency and throughput of forensic DNA analysis.