



### **B161 An Automated System for the High-Throughput Processing Of Differential Extraction Samples**

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After attending this presentation, attendees will understand the process by which the Differex™ system allows for the semi-automated separation of sexual assault samples into sperm and epithelial fractions using a liquid-handling robot.

This presentation will impact the forensic community by introducing a process that will allow numerous differential extractions to be performed in a single work day.

Differential extraction is the method by which spermatozoa are separated from epithelial cells present in sexual assault samples in preparation for STR amplification of the respective fractions. The technique in common usage employs a selective proteinase K lysis to liberate DNA from the epithelial cells of the sample while leaving the sperm heads intact. An initial centrifugation, following proteinase K digest, pellets sperm to the bottom of the digest tube. The supernatant is removed from the digest tube and retained for downstream processing as an epithelial fraction. The sperm pellet is subjected to a serial washing process to dilute and remove epithelial DNA-containing supernatant. The sperm pellet then undergoes a second digest step in the presence of dithiothreitol as a reducing agent to liberate sperm DNA for downstream processing as the sperm fraction. The core limitations of this commonly used method are: it is both time and labor intensive, it is low-throughput, and it produces a quality of result that can vary greatly from day-to-day or analyst-to-analyst.

The Automated Differex™ System combines Promega's Differex™ and DNA IQ™ system reagents in a novel pellet-capping process which allows for high-throughput, semi-automated differential extraction of sexual assault samples. Following proteinase-K digest, the starting point for Automated Differex™ separation is a plate of sperm pellets and epithelial digest supernatants resulting from centrifugation in a SlicPrep96® apparatus. Automated Differex™ uses DNA IQ™ Resin paramagnetic particles as part of a pellet-capping process to restrain sperm bodies allowing for supernatant manipulation without pellet disruption. After washing, Differex™ Separation Solution is used to float the majority of residual epithelial DNA-containing wash buffer away from the pellet for removal without disturbing the pellet itself. A dithiothreitol sperm-lysis step liberates sperm DNA from the pellet. Sperm and epithelial fractions of each sample then undergo automated nucleic acid isolation using the DNA IQ™ System.

The Automated Differex technology's pellet-capping process allows for the uniform and high-throughput processing of differential extraction separations. The incorporation of DNA IQ™ technology into Automated Differex™ provides high-quality nucleic acid purification from both sperm and epithelial fractions. Methods for the Automated Differex™ System will be available for the Beckman Coulter™ Biomek®2000 and Biomek®3000 Laboratory Automation Workstations, as well as for the Tecan Freedom EVO® Workstation. The coupling of Automated Differex™ separation and DNA IQ™ nucleic acid purification methods will allow analysts to go from sexual assault sample to purified fraction eluates in less than 3 hours for 8 samples, and in less than 5 hours for 48 samples. Detailed descriptions of the automated methodologies will be presented. Data will also be presented demonstrating the Automated Differex™ System's sensitivity and quality of separation using mock sexual assault samples comprising sub- microliter equivalents of semen in vaginal epithelial backgrounds.

**Forensic Science, Differential Extraction, Automation**