

## **VALIDATION OF A FULLY AUTOMATED STANDARD DIFFERENTIAL EXTRACTION PROCESS ON THE HAMILTON MICROLAB® AUTOLYS STAR SYSTEM**

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Sexual assault evidence comprises a significant portion of the samples that are analyzed in forensic DNA laboratories. This evidence, which is most often a mixture of female epithelial cells and male sperm cells, typically undergoes a differential extraction procedure to separate the mixture into sperm and non-sperm fractions, and each fraction is then analyzed by autosomal short-tandem repeat (STR) genotyping. A standard differential procedure (Nature 1985: 318: 577-579) consists of a mild initial lysis step to preferentially digest the non-sperm (epithelial) cells, followed by multiple centrifugation/wash steps to remove any remaining non-sperm fraction DNA and to isolate the undigested sperm cell pellet; each fraction is then extracted separately for STR typing. This manually intensive procedure has been difficult to fully automate due to the variety of sample manipulations required (incubation, substrate removal, shaking, washing, centrifugation). We describe here the validation of a fully automated standard differential procedure using the Hamilton AutoLys STAR liquid handling system with the co-developed "AL-STAR" script, which processes up to 24 samples in a single automation run. This is a continuation of work previously published (FSIG 2018: 34: 170-177, FSIG 2019: 40: 96-104) and includes studies on sensitivity, precision, reproducibility and repeatability, contamination, varied substrates, and mock SAE case samples. The sensitivity studies were performed on vaginal swabs spiked with varying quantities of semen and included side-by-side manual differential extractions on identically prepared swabs for the lower, more challenging quantities. The varied substrates included cuttings of swabs, panties, panty liners, and proficiency fabric swatches. The mock SAE case samples included post-coital vaginal and scrotal swabs. Results of each study demonstrate this process is fit for purpose and will be presented.