# ENHANCED PERFORMANCE OF THE PROMEGA DIFFEREX PROTOCOL IN COMBINATION TO THE COPAN NAO BASKET FOR SEMINAL FLUID DNA PROFILING FROM VAGINAL SPECIMENTS OF SEXUAL ASSAULT CASE WORK

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Sexual assault specimens processing in forensic DNA laboratory, especially vaginal swabs collected from victims by medical personnel, have to overcome two major problems: one is the large amount of female cellular material that makes unclear the presence of male perpetrator not allowing identification with autosomal STR systems; the second one is the use of cotton swabs, which are not fully suitable for subsequent analysis in the laboratory. The differential extraction approach, which physically separates the female epithelial cells from the male spermatozoa, allows genetical characterization of male fraction using autosomal markers. The Promega Enhanced Differex™ (ED) protocol is simple and rapid compared to the classic method (Gill et al.) The goal of this study was to validate the performance of the Copan NAO (Nucleic Acid Optimizer) basket with the Promega ED protocol by:

1. Performing the sample lysing step directly in the NAO.
2. Eliminating the need of transferring the sample after the first lysis step optimizes DNA recovery and drops probability of contamination.

For this study simulated vaginal swabs (SVS) were prepared using epithelial cells (EC) from female buccal swab and donor seminal fluid (SF). EC aliquots, inoculated with 50 and 20ul each of undiluted and 1:5, 1:25, 1:125, 1:250 , 1:500 and 1:1000 dilutions of SF, were spotted on FLOQSwabs™ in triplicates. FLOQSwabs™ spotted with 50ul and 20ul of EC and SF were prepared as controls. SVS and controls were processed using the ED+NAO protocol on the Maxwell on LEV Forensic mode. DNA quantification of the extracts was done with Promega PowerQuant kit on the Real-Time -PCR System ABI 7500 HID. Normalization and amplification was performed with Promega PowerPlex Fusion 6C on an ABI 9700 Thermal Cycler, followed by capillary electrophoresis sequencer on an AB 3500XL.

Full and high-quality profiles with elevated peak heights were generated from all samples. The data obtained demonstrated that the NAO+ ED protocol can be used for differential DNA extraction of spermatozoa in vaginal samples. Highly purified sperm pellets were obtained with clean genotypic profiles and high PHR, often greater than 90% even with SVS with highly diluted (1:1000) SF. NAO+ ED protocol seems help to reduce accidental contamination and it is advisable for sexual assault caseworks.