B123 The Optimization of a Semi-Automated Differential Extraction Protocol for Recovery of Low-Level Male DNA From Sexual Assault Samples

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Learning Overview: After attending this presentation, attendees will better understand the challenges associated with the extraction, isolation, and purification of sexual assault samples in which a low-level of male DNA is in the presence of an excess of female DNA. Attendees will also learn techniques for the successful recovery of purified male DNA using a combination of manual separation methods and automated extraction platforms to generate robust, highly-discriminatory STR profiles.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a highly efficient workflow solution for lower-throughput forensic laboratories to maximize the amount of easily interpretable discriminating genetic information which can be produced from sexual assault samples in which the male-to-female mixture ratio is in extreme proportions.

Often, sexual assault reporting is delayed, which can result in diminished quantities of male DNA being recovered off intimate swabs collected from the victim. This can subsequently lead to minimal success in the generation of an autosomal STR profile of the male perpetrator. With an excess of female DNA going into the extraction process, it is possible that not all the epithelial cells are being digested during the initial incubation. The residual intact or partially digested female DNA epithelial cells would then be pelleted with the sperm cells and carried forward through extraction and downstream analysis. Although Y-STRs may be a suitable alternative for these types of samples, application of this technology results in a decrease of discriminatory power in identifying the perpetrator.

The goal of the current research was to optimize the front-end manual separation of seminal and epithelial fractions, to be incorporated into a semi-automated differential extraction workflow using a robotic DNA extraction platform, with the end-goal of reducing the amount of female epithelial cells being carried over into the seminal fraction without increasing the amount of hands-on time required by the analyst. To do so, it was imperative to assess the differences in quantitative and qualitative performance of samples which underwent variable numbers of sperm pellet washes and to assess the effects of a single epithelial cell lysis step compared to two rounds of epithelial cell lyses. Preliminary data generated from vaginal swabs spotted with neat semen showed that increasing the number of wash steps and incorporation of a secondary epithelial lysis step both decreased the total quantity of human DNA recovered without negatively impacting the quantity of male DNA recovered in the seminal fraction.

The reliability of the previously discussed methods was also assessed with extreme male-to-female mixture ratios of 1:1000 neat seminal fluid to homogenized vaginal fluid. The number of wash steps was first assessed, which resulted in the addition of a second wash step decreasing the amount of total human DNA present ($\bar{x}_1$ = 0.0749 ng, $\bar{x}_2$ = 0.0273 ng), as seen previously. However, without visualization of a sperm pellet, the addition of a second wash step also drastically decreased the amount of male DNA present ($\bar{x}_1$ = 0.0703 ng, $\bar{x}_2$ = 0.0183 ng) and subsequently negatively affected the post-extraction male-to-female ratio, resulting in a more convoluted STR analysis. When paired with a single wash step, the incorporation of the secondary epithelial lysis step resulted in statistically significant differences in the amounts of total human and male DNA recovered from the seminal fraction compared to the usage of a single epithelial lysis step (F_{SA} = 11.723; df = 1, 19; p = 0.00303 and F_Y = 15.563; df = 1, 19; p = 0.000949), resulting in easily resolvable, highly discriminatory single source or male major contributor profiles.

This study demonstrates that increasing the number of wash steps applied to a sperm pellet during a differential extraction can be deleterious for sexual assault samples in which the male-to-female mixture ratio is in extreme proportions, due to loss of sperm fraction DNA during the additional wash steps. This study also demonstrates that utilization of a secondary lysis step is extremely beneficial to the production of isolated, highly-purified seminal fractions for further extraction utilizing a robotic extraction platform. Further work is being completed to assess any possible effects of the proposed method on analysis of the epithelial fraction as well as performance of the method with more extreme mixture ratios out to 1:5000.

Differential Extraction, Method Optimization, Robotic DNA Extraction