B31 Development of an Improved Cell Elution and Preferential Lysis Method for Sexual Assault Cotton Swab Evidence Analysis

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The focus of this project is the development of an improved method for the elution and preferential lysis of cells from cotton swab evidence samples collected from sexual assault victims.

This presentation will impact the forensic community by demonstrating an alternative to conventional differential extraction (DE) for increased recovery of biological materials in an effort to increase the amount of male DNA available for genetic identification.

Genetic analysis of mixed profile DNA samples obtained from vaginal swabs is a well-established technique in the investigation of sexual assault and rape cases. Unfortunately, the procedures involved in a typical forensic DNA analysis require that significant laboratory time be dedicated to a single case, particularly regarding sample preparation. Because of time and funding constraints involved in the investigation of such cases, a significant backlog exists in many DNA analysis laboratories.

The current protocol for recovery of genetic material from cotton swabs involves DE, a method that utilizes anionic detergent and proteinase K to selectively lyse vaginal epithelial cells while retaining intact sperm cells. The female DNA released into solution from the lysed vaginal epithelial cells is separated from intact sperm cells via centrifugation. The DE method does not always provide for efficient independent genetic analysis of the separated male fraction. Although this treatment decreases the number of vaginal cells present in the sperm cell suspension, sperm cell lysis and subsequent loss of valuable evidential material often occurs during the proteolytic digestion step of the traditional DE process.[1] In addition, the method is time-consuming, often requiring overnight incubation of a swab sample.

Microchip technology offers a rapid, cost-effective alternative to conventional DNA analysis methods, and separation of sperm and epithelial cells on a microchip as the first step in forensic analysis of sexual assault evidence has been demonstrated.[2] This method required intact sperm and epithelial cells; therefore, a method for release of intact cells from cotton swabs was developed.[1, 3] This work showed increased recovery of sperm cells over traditional DE methods (two- fold), increasing the likelihood of obtaining a perpetrator genetic profile, particularly with low copy number (LCN) samples. Use of this cell recovery method with the traditional analysis requires selective lysis of the epithelial cells without loss of sperm cells. Whether associated with conventional processing or an anticipated lab-on-a-chip platform, increased sperm cell recovery and comprehensive epithelial cell lysis would be beneficial to the forensic community.

The presented work will detail the improved method for cellular recovery from swab samples along with a method for preferential lysis of epithelial cells. Elution of cells from cotton swabs utilizes an anionic detergent solution and allows recovery of intact sperm and epithelial cells, which can be separated to provide male and female fractions. Preferential lysis of the collected epithelial cells is performed using proteolytic digestion of cell solution, providing a starting point for traditional processing, but with a greater recovery of sperm cells. Various cell elution and preferential lysis conditions were evaluated by counting the eluted sperm and epithelial cells using a hemacytometer. Results indicate that an optimized two-step elution/preferential lysis method required less time than conventional DE, provided comprehensive lysis of epithelial cells to decrease contamination of the male fraction, and improved the recovery of sperm cells.

References:

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Differential Extraction, Preferential Lysis, Cell Elution

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