B20 Enhancement of Sperm Cell Recovery From Cotton Swabs for Rape Kit Analysis: Anionic Detergent-Mediated Cell Elution in the Absence of Proteinase K

Jessica C. Voorhees, MS, Kate Manning, Jerome P. Ferrance, PhD, and James P. Landers, PhD, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904*

After attending this presentation, attendees will have learned about an improved method for the elution of cells from a cotton swab evidence sample collected from a sexual assault victim.

This presentation will impact the forensic community and/or humanity by demonstrating an alternative to conventional differential extraction for increased recovery of biological materials from cotton swabs in an effort to improve recovery of sperm cells for DNA extraction and genetic identification.

The focus of this project is the development of an improved method for the elution of cells from a cotton swab evidence sample collected from a sexual assault victim. The procedure incorporates a detergent solution for increased recovery of biological materials from cotton swabs, in an effort to improve recovery of sperm cells for DNA extraction and 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 in the sample preparation steps. 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, known as differential extraction (DE), involves significant sample handling and is time-consuming, often requiring overnight incubation of a swab sample. The solution used for DNA recovery from swabs includes proteinase K and a detergent, the combination of which selectively lyses epithelial cells while eluting sperm cells intact. Centrifugation pellets the sperm cells, separating them from female DNA in solution from the lysed vaginal epithelial cells. 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. The DE method, therefore, does not always provide for efficient independent genetic analysis of the separated male fraction.

Microchip technology offers a rapid, cost-effective alternative to conventional DNA analysis methods. Techniques performed on microchips are particularly advantageous because they can be integrated with downstream analytical steps on a single microfluidic device in the form of a lab-on-a-chip device. Implementation of integrated systems for forensic DNA analysis will reduce analysis times, and, therefore, the forensic casework backlog. However, traditional isolation of separate sperm and epithelial DNA fractions using DE incorporates centrifugation steps, which are not easily implemented on a microchip. Microchip methods for isolating intact male and female cells have been reported[1,2], but the overall effectiveness of the procedure is ultimately dependent on the efficiency with which material can be eluted and recovered from a cotton swab.

Previous studies have shown that intact sperm and epithelial cells can be recovered from swab samples using enzymatic digestion of the cotton matrix[3]; this method, however, did not enhance sperm cell recovery over the traditional DE buffer. Current studies focus on treatment of the swab samples with anionic detergents to enhance sperm cell recovery, and show that a considerable fraction of sperm cells are lysed by the proteolytic digestion utilized in the conventional DE method.[4] To optimize cellular elution conditions, several detergents were evaluated, with the sperm and epithelial cells eluted from each cotton swab sample counted using a hemacytometer. Results indicate that elution using anionic detergents over neutral or cationic detergents improved the recovery of sperm cells in less time than required for conventional DE. Optimum cellular elution conditions using detergents will be presented. In addition, information regarding the development of alternative preferential lysis methods will be discussed.

References:

1 Horsman KM, Barker SLR, Ferrance JP, Forrest KA, Koen KA, Landers JP. Anal Chem 2005;77:742-749.

2 Peat M. Acoustic differential extraction: a novel alternative to conventional differential extraction; 2006 Feb 20-25; Seattle. American Academy of Forensic Sciences, 2006 Feb 20-25.

3 Voorhees JC, Ferrance JP, Landers JP. J Forensic Sci 2006;51(3):574- 579.

4 Voorhees JC, Manning K, Linke SJ, Ferrance JP, Landers JP. 2006; in progress.

Cell Elution, Anionic Detergent, Differential Extraction

 ** Presenting Author*