B7 Enzyme-Mediated Digestion of Cellulose and Pectin for Enhanced Cell Elution of Sperm Cells From Cotton Swabs

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

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 used by law enforcement agencies for recovery of cellular material from a cotton matrix involves significant sample han- dling. Furthermore, it is a time-consuming process, often requiring overnight incubation of a swab sample in buffer that aids in optimal DNA recovery. The extraction solution used in the recovery of DNA from swabs includes proteinase K and a detergent, the combination of which selectively lyses the fragile epithelial cells while eluting sperm cells intact. The solution is then centrifuged to pellet the sperm cells, separating them from the solution containing the DNA from vaginal epithelial cells, allowing independent genetic analysis of male and female DNA.

The time required for forensic DNA analysis can be greatly reduced by performing the electrophoretic separation on microfabricated glass devices. The speed and efficiency of separations on microdevices provide benefits over both conventional slab gel and capillary electrophoretic sep- arations. In addition, these devices allow for the integration of other pro- cessing steps, including sample preparation methods. Isolation of separate sperm and epithelial DNA fractions using traditional differential extraction methods requires centrifugation, which is not easily implemented on a microchip; however, a microchip method for isolating male and female cells has been reported[1] which allows independent DNA extraction from the separate cell types. This method relies on recovery of intact cells from sample swabs, therefore, a cell desorption process that reduces extraction time and leaves the epithelial cells intact would be advantageous for devel- oping genetic analysis on a micro-total analysis system (μ-TAS). If this cell elution method could also increase the number of sperm cells recovered from swabs over differential extraction, this method would provide addi- tional benefits in increasing the amount of perpetrator DNA recovered for analysis.

Constituents of cotton include cellulose, a polysaccharide that com- poses the cotton fibers, and pectin, which acts as a surface adhesive between neighboring plant cells. Microscopic examination of a cotton swab on which a semen sample had been applied and allowed to dry suggested that sperm retention on the swab was due to adhesion of sperm cells on the surface of cellulose strands. Previous studies have shown that enzymes that digest cellulose reduce the time required for sperm and epithelial cells to be released from the swab into solution.[2] In an effort to optimize cellular elution conditions, enzymes that digest either the cellulose or the pectin components of the swab were evaluated separately and in combination. In addition, the effects of adding detergent after enzyme treatment were investigated. Sperm and epithelial cells eluted from each cotton swab sample were counted using a hemacytometer. Results indicate that elution using enzymes improved the recovery of sperm cells without lysing epithelial cells, and sperm cell desorption using a combination of enzyme and detergent is greater than that seen with current elution methods. Optimum cellular elution conditions using the enzymes cellulase and pectinase will be presented. In addition, information regarding the development of a receptacle that interfaces a cotton swab sample with a μ- TAS on a microfabricated glass device will be discussed.

The procedure incorporates enzymes for digestion of the cellulose matrix, resulting in the removal of intact cells, in an effort to improve recovery of sperm cells while circumventing conventional differential extraction. If this cell elution method could increase the number of sperm cells recovered from swabs over differential extraction, it would provide additional benefits in increasing the amount of perpetrator DNA recovered for analysis.

References:

1. Horsman, K.; Barker, S.L.R.; Ferrance, J.P.; Forrest, K.A.; Koen, K.A.; Landers, J.P. *Anal Chem* 2005, 77, 742-749.
2. Voorhees, J.C.; Ferrance, J.P.; Landers, J.P. *Journal of Forensic Science.* 005, Submitted.

Cell Elution, Enzymes, Differential Extraction

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