A18 Analysis of Mock Sexual Assault Samples Using a One-Step Cell Elution and Preferential Lysis Method

Jenny A. Lounsbury, MSFS, and Shanti Nambiar, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904; Robert C. Giles, PhD, Orchid Cellmark, Incorporated, 13988 Diplomat Drive, Suite 100, Farmers Branch, TX 75234; and James P. Landers, PhD, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904*

After attending this presentation, attendees will have gained an understanding of the improvements that have been made to a one-step cell

elution and preferential lysis method for the recovery of cellular material from cotton swabs.

This presentation will impact the forensic science community by describing a fast and simple method to recover sperm cells from low cell number samples, which could help increase sexual assault sample throughput.

Differential extraction is the most common method for the recovery and separation of male and female cellular material from sexual assault swabs, accomplished by utilizing proteinase K and an anionic detergent. However, differential extraction is laborious and time-consuming, often requiring an overnight incubation of the cotton swab. In addition, some sperm cells are lost due to digestion by proteinase K, which can greatly decrease the likelihood of obtaining a STR profile of the perpetrator[1].

Microfluidic devices can be used for forensic analysis, providing a rapid, cost-effective alternative to the most widely used DNA analysis methods. Furthermore, these devices allow for the potential to integrate multiple analysis processes on a single device.[2] A microfluidic device has been used in combination with an acoustic trapping method to separate sperm cells from female epithelial cell lysate;[3] however, results obtained with this method depend on the recovery of sperm cells from the cotton swab, as well as comprehensive lysis of epithelial cells. A faster, reliable method for the successful recovery of sperm cells and lysis of epithelial cells from a sample is necessary to further benefit from these novel examples of integrated microfluidic devices for DNA analysis of sexual assault evidence.

Previous work has demonstrated a two-step method consisting of a thirty-minute incubation to elute all cellular material, followed by the addition of proteinase K and a second thirty-minute incubation to selectively lyse female epithelial cells[4]. This two-step method provides a comprehensive lysis of female epithelial cells and nearly doubles the recovery of sperm cells over differential extraction techniques. However, more recent work has improved the two-step method by using an Orchid Cellmark proprietary reagent and a single step method to reduce incubation time to as little as thirty minutes, while providing comparable sperm cell recoveries to those obtained with the two-step method[4].

The current work and focus of this presentation is on the optimization of the one-step method to maximize the recovery of sperm cells from mock sexual assault samples and the application of this method to low cell number mock sexual assault samples. Low cell number samples are defined in this work as samples containing ≤3000 sperm cells. Several incubation times and temperatures were evaluated to maximize recovery of sperm cells and the comprehensive lysis of female epithelial cells. Additionally, several anionic detergents were evaluated to determine if sperm cell recovery could be increased. The sperm and epithelial cells eluted from mock sexual assault evidence swabs were counted using a hemacytometer and results indicate that sperm cell recovery can reach as high as 90% with a thirty-minute incubation using the one-step method. This method has the potential to be used as an alternative to conventional differential extraction methods, as well as being adapted to microfluidic devices for eventual integration into a micro-total analysis system for DNA processing and more rapid analysis of sexual assault samples.

References:

1 Norris, JV, Manning K, Linke SJ, Ferrance JP, Landers JP. J Forensic Sci 2007;52(4):800-805.

2 Easely, CJ, Karlinsey, JM, Bienvenue, JM, Legendre, LA, Roper, MG, Feldman, SH, Hughes, MA, Hewlett, EL, Merkel, TJ, Ferrance, JP, Landers, JP. 2006:103(51):19272-19277.

3 Norris, JV, Evander, M, Horsman-Hall, K, Nilsson, J, Laurell, T, Landers, JP., Anal Chem 2009:Article ASAP.

4 Norris, JV, Cunniffe, H, Manning, K, Linke, SJ, Ferrance, JP, Landers, JP. Development of an Improved Cell Elution and Preferential Lysis Method for Sexual Assault Cotton Swab Evidence Analysis; Washington, DC. American Academy of Forensic Sciences, 2008 Feb 18-23.

5 Lounsbury, JA, Norris, JV, Cunniffe, H, Giles, RC, Landers, JP. Development of a One-Step Cell Elution and Preferential Lysis Method for Analysis of Sexual Assault Samples; Denver, CO. American Academy of Forensic Sciences, 2009 Feb 16-21.

 ** Presenting Author*

Cell Elution, Preferential Lysis, Low Cell Number

 ** Presenting Author*