B104 Why Perform Y-Screening? Validation of a Novel Sperm Lysis Protocol to Improve Sexual Assault Triaging

Victoria Hsieh, 140 W 69th Street, Apt 85B, New York, NY 10023; David R. Fisher, MS, NYC OCME, Dept of Forensic Biology, 421 E 26th Street, #11-50, New York, NY 10016; Robin W. Cotton, PhD, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, R 806, Boston, MA 02118; Jeffrey A. Hickey, MS, 1505 Rutledge Avenue, Charlottesville, VA 22903-1417; Kirsty Mayall, MS, Microlab Inc., 705 Dale Avenue, Charlottesville, VA 22903; Orion Scott, PhD, 705D Dale Avenue, Charlottesville, VA 22903; David J. Saul, PhD, ZyGEM NZ Ltd, Innovation Park, Ruakura Road, Hamilton, Waikato 3216, NEW ZEALAND; Delphine Le Roux, PhD, University of Virginia, Dept of Chemistry at UVA, McCormick Road, PO Box 400319, Charlottesville, VA 22901; Jacquelyn A. DuVall, BSc, University of Virginia, Dept of Chemistry, UVA, McCormick Road, PO Box 400319, Charlottesville, VA 22904; and James P. Landers, PhD, University of Virginia, Dept of Chemistry, McCormick Road, Charlottesville, VA 22904*

After attending this presentation, attendees will better understand the process of screening sexual assault evidence using a novel sperm lysis protocol and commercially available quantitative Polymerase Chain Reaction (qPCR) kits.

This presentation will impact the forensic science community by demonstrating a method for triaging sexual assault kits by rapidly lysing sperm cells, allowing for the detection of male DNA with Y-specific qPCR.

Many laboratories in the United States are making efforts to reduce backlogged sexual assault cases and/or reduce the processing time for incoming cases. These laboratories often screen large amounts of evidence, looking for the best samples to test in order to get a perpetrator's DNA profile. This often involves presumptive tests for semen, amylase, or blood. Some laboratories have begun to use qPCR as a screening tool for male DNA since most sexual assault cases have a male suspect. Consequently, there is demand for a quick sperm lysis protocol, which would allow for rapid high-throughput screening of potential sexual assault evidence.

Typically, DNA is released from sperm using a combination of detergents and reducing agents, which can be inhibitory to downstream assays, such as PCR and real-time PCR. Most sperm lysis procedures require additional steps to remove these potentially inhibitory reagents from the DNA extract. These clean-up procedures add more transfer steps, which lengthen the process and can add cost. In addition to the time and cost considerations, extra transfer steps can lead to sample switching, contamination, or loss of DNA.

This presentation will describe the validation of the ZyGEM® PDQeX for DNA extraction, including sperm lysis. This instrument uses a novel protocol and reagents for sperm lysis. The end result is DNA ready for qPCR or for Short Tandem Repeat (STR) profiling without additional clean-up steps. Data from the developmental validation of the manufacturer, as well as data from the internal validation of this system by the New York City Office of Chief Medical Examiner, will be presented.

The validation data includes sensitivity, precision and accuracy, mixture, contamination, and known and non-probative studies. This data has shown that the system can lyse sperm mixed with overwhelming quantities of female epithelial cells. Sensitivity studies have illustrated that male/female mixtures containing as few as one sperm per microliter can be lysed and then detected and quantified using qPCR. Mock casework and stability studies have shown that the system can extract DNA from realistic and challenging mock casework samples, such

as mock vaginal swabs and sperm deposited on denim and other fabrics. The qPCR data was also used to accurately estimate the amount of template DNA for STR amplification with commercially available kits. The quantification data was found to be a good estimate for the purposes of STR amplification, indicating that the DNA extracts were not inhibiting qPCR or STR amplification.

This data will demonstrate that this system can extract DNA from typical sexual assault evidence that is ready for commercially available qPCR and STR kits. This process enables laboratories to use Y-specific qPCR to rapidly screen sexual assault kits for useful evidence. The screening method is an efficient way for laboratories to triage samples with sufficient male DNA to pursue STR analysis.

Y-Specific qPCR, Sperm Lysis, Male Screening