B128 A Method for Optimizing Pressure-Based Extraction and Direct Polymerase Chain Reaction (PCR) of Simulated Sexual Assault Samples

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After attending this presentation, attendees will better understand a novel method for differentiating, extracting, and amplifying epithelial and sperm mixture samples.

This presentation will impact the forensic science community by providing results for a method that can decrease the analysis time for sexual assault samples. Attendees will see results of a new method to aid in direct PCR for amplification of simulated sexual assault samples. These results will add to previous work performed using pressure cycling by demonstrating a method to eliminate the long cell lysis and purification process as well as coupling these methods with direct PCR.

In this study, pressure cycling is used to prepare differentiated lysates of epithelial and sperm mixtures for rapid analysis. It is widely known that many Sexual Assault Kits (SAKs) currently await processing. For example, in January of 2016, more than 13,400 SAKs had been reported as not being analyzed in the state of Florida.[1] This study presents a method for faster analysis of these types of samples. Epithelial and sperm mixtures often require large amounts of time and effort to differentiate the fractions. The goal is to develop a rapid STR-based screening that eliminates the cell lysis, purification, and quantitation steps for a faster analysis.

This study involved the use of alkaline lysis and direct PCR to extract and amplify epithelial and sperm DNA from simulated SAK samples, with various concentrations of epithelial and sperm cells. The mixtures that were tested ranged from 1:1 to 20:1, epithelial: sperm. The first part of the project consisted of the optimization of pressure cycling and alkaline lysis for simulated sexual assault samples. Concentrations of Sodium Hydroxide (NaOH) and temperature were tested, as well as pre-pressure steps. Concentrations of 0.4N, 0.3N, 0.2N, and 0.1N NaOH were used to determine an increase in epithelial recovery. Immunomagnetic cell capture prior to the Pressure Cycling Technology (PCT) step was used to increase the differential recovery of epithelial and sperm fractions. This technique utilizes the EasySep™ Human EpCam Positive Selection Kit to capture epithelial cells with antibodies that target epithelial cell receptors. These are then attached to dextran-coated magnetic particles added to the sample. Once the sample tube was inserted into a magnet, excess epithelial cells were trapped on the sides of the tube. In the second part of the project, the simulated sexual assault samples were processed with pressure cycling and a direct PCR protocol was developed. Cotton swabs with epithelial and sperm cells were treated at room temperature with 0.2N NaOH in a PULSE™ tube. In the same tube, pressure cycling was performed with the optimized protocol using the Barocycler® NEP 2320; this step was repeated and the epithelial lysate was neutralized. Then the swab was treated again with alkali and heated to 95°C to lyse the sperm cells. These were also neutralized and the cotton swab removed. Both lysates were amplified with direct PCR using a 7-locus primer set on the Applied Biosystems GeneAmp® PCR System 9700 and/or amplified with rapid/direct PCR on a Streck Philisa® Thermal Cycler. Amplification conditions and speed were optimized by examining the effect of primer, polymerase, buffer, and dNTPs. The STR multiplex was then analyzed on an Applied Biosystems® ABI PRISM®

310 Genetic Analyzer to assess the quality of the mixture separation.

The results demonstrate the potential of alkaline lysis and pressure cycling to greatly expedite the analysis of differential extraction. In regard to optimization of the pressure cycling, the use of 0.2N NaOH resulted in improved recovery and removal of epithelial cell DNA from the substrate, 115% compared to the unmodified protocol, according to data obtained from quantification with Plexor® HY. Genetic profiles from the ABI PRISM® 310 Genetic Analyzer revealed a slight majority male profile for the sperm fraction at a 20:1 female epithelial cell to sperm cell ratio. The addition of the immunomagnetic cell capture treatment prior to the PCT step produced a clear majority male profile with female allelic dropout. The addition of faster amplification to this protocol can greatly increase the speed of the results obtained.

Reference(s):

1. Florida Department of Law Enforcement. *Assessment of unsubmitted sexual assault kits: Executive summary*. 2016.

Direct PCR, Pressure Cycling Technology, Simulated SAK Samples