B13 The Development and Optimization of a Direct Polymerase Chain Reaction (PCR) System of Mixtures of Sperm and Epithelial Lysates From Cotton Swabs

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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 mixture samples of sperm and epithelial cells. Attendees will observe the results of an experimental design-based method that aids in direct PCR for amplification of post-coital lysates. These results provide a method to minimize the long cell lysis and purification process by quickly extracting and directly amplifying cell lysates.

At present, there is increasing concern regarding case backlogs involving Sexual Assault Kits (SAK). The goal of this project is to improve the speed of extraction and to provide a method for quickly separating and screening mixed samples. It is common for epithelial and sperm cell mixtures to require labor-intensive processes and time to achieve differential extraction. The goal is to develop a rapid Short Tandem Repeat (STR) -based screening method that minimizes the DNA extraction, purification, and quantitation steps for a faster analysis.

In this study, alkaline lysis and direct PCR were used to extract and amplify epithelial and sperm DNA from simulated SAK samples, with various concentrations of epithelial and sperm cells. The direct PCR method was developed and optimized using an experimental design software program using mixture design methods. Cotton swabs were treated with 0.05N sodium hydroxide (NaOH) under ten cycles of 15 seconds at 20kpsi and 15 seconds at ambient pressure. This removed and lysed epithelial cells from the cotton swab. The swab then underwent incubation in 0.4N NaOH at 95°C for five minutes to remove and lyse sperm cells. After neutralization, the lysate was incubated at 50°C for ten minutes with 125mM dithiothreitol (DTT) to remove the protamines, isolating the male DNA. The samples underwent drop dialysis and were amplified using an inhibition-resistant polymerase. The PCR reaction mixture was optimized using experimental mixture designs. The experimental design considered salt concentration, buffer addition, deoxynucleotide triphosphate (dNTP) addition, primer addition, and PCR enhancers to develop experiments to maximize amplification of sperm and epithelial DNA. Output was measured by peak height of Amelogenin, D5, D13, D7, D16, CSF, and Penta D. The STR multiplex was analyzed using capillary electrophoresis and peak height was evaluated to determine the most effective reaction mix.

Results demonstrate the most effective amplification of sperm and epithelial DNA lysates without isolation and purification. The alkaline and pressure lysis effectively separate the epithelial fraction from the sperm, with the sperm being further lysed with heat, alkali, and reduction. The experimental runs demonstrate the enhancement of direct PCR using combinations of the reaction components. The analysis of the experimental runs is accomplished using mixture design analysis with experimental design software. This uses the results to model a contour plot demonstrating the expected ideal reaction mix to maximize peak heights. The model is tested with more experimental runs based off the components that lead to increased peak heights to determine the ideal conditions for maximized peak heights.

Direct PCR, Experimental Design Methods, Pressure Cycling

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