A108 Application of Pressure Cycling Technology (PCT) in Differential Extraction

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After attending this presentation, attendees will understand a new method for the differential extraction of DNA from sperm and epithelial cells in sexual assault casework.

This presentation will impact the forensic community by providing a better understanding of how pressure cycling technology can be used to speed up and simplify the extraction process.

One of the stumbling blocks in obtaining a successful male genetic profile in sexual assault cases involves the separation of the evidence left behind by the perpetrator from that of the victim. The conventional differential extraction methods used for the separation of DNA from sperm and epithelial cells is time consuming and requires expertise. It is imperative to develop a method that addresses the issues of time, efficiency, and ease of use.

Pressure cycling technology sample preparation system (PCT SPS) is a novel method that involves the use of pressure to disrupt tissues, cells, and cellular structures enabling the recovery of their components. In this research, the authors utilized a commercially available instrument from Pressure Biosciences with a hydrostatic pressure chamber that generates alternating cycles of ambient and high pressure up to 35,000 psi resulting in the lysis of cells. Sample cells were placed in liquid suspension in microtubes and subjected to a range of on and off pressure pulses in an

attempt to isolate and recover DNA. The microtubes were made from a fluoropolymer that renders them chemically resistant to improve sample recovery and limit adsorption.

The current study involves the application of pressure cycling technology in the extraction of nucleic acids from sperm cells and vaginal epithelial cells. The cells were suspended in 1X PBS buffer (pH 7.4) and subjected to 5,000 psi- 35,000 psi pressure in increments of 5,000 psi, accompanied by varying number of cycles in order to determine the conditions at which one type of cell could be lysed differentially over the other. Samples were placed in microtubes and introduced into the pressure chamber. This pressure treatment was followed by phenol chloroform isoamyl alcohol purification to obtain a clean DNA sample devoid of salts and proteins for successful downstream analysis. The purified DNA was quantified with an Alu-based real-time PCR method using SYBR green.

The initial studies indicate the potential of PCT application in analyzing samples from sexual assault cases, in particular, indicating improved extraction of sperm DNA at high pressures when compared to epithelial cells. Overall these results provide new opportunities to explore the ability to generate male DNA profile by selectively lysing sperm cells from mixtures.

Differential Lysis, Pressure, Sexual Assault

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