## A73 Application of Pressure Cycling Technology (PCT) in Differential Extraction

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After attending this presentation, attendees will understand the principles behind a rapid and efficient method of extracting sexual assault evidence using a pressure-based protocol that is designed to burst open and extract DNA from male sperm cells in rape kits or other mixed forensic stains.

This presentation will impact the forensic science community by providing a better understanding of how the application of pressure pulsing can be used for more specific detection of individual cells that will enable rapid and selective processing of sexual assault evidence.

Separating the sources of DNA from different contributors to a stain reduces the difficulty associated with mixture analysis and data interpretation. The processing and interpretation of such mixed DNA samples has long been recognized as a bottleneck in forensic DNA analysis. Organic differential extraction is the most commonly used method to isolate sperm DNA from sexual assault evidence. This two-step extraction procedure involves selective digestion of epithelial cells in the first step, followed by isolation and digestion of sperm cell pellet. The major disadvantages of this technique are incomplete separation of sperm and non-sperm fractions, particularly in samples that are overwhelmed by large numbers of female epithelial cells relative to sperm cells, and the time-consuming nature of the process.

The objective of the study was development of a method using Pressure Cycling Technology (PCT) combined with reagents to selectively disrupt sperm or epithelial cells and recover DNA. The extraction procedure is performed utilizing the Barocycler® NEP 2320, a commercially available instrument from Pressure Biosciences, Inc., equipped with a hydrostatic pressure chamber that generates alternating cycles of ambient and high pressures with a range of 5 to 45 kpsi. Samples such as cotton swabs or cuttings of cloth can be directly extracted using this technique by simply placing them in a pressure cell along with an appropriate buffer.

The current study involves the application of pressure cycling technology in the selective digestion of sperm cells from evidence mixtures collected from different substrates with an emphasis on the role of buffer composition on sperm DNA yields and increase in selectivity of extraction. The cells were extracted into 1X PBS buffer (pH 7.4) with varying buffer compositions and subjected to a pressure of 45,000 psi. 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 Promega Plexor® HY system followed by an STR analysis using Promega PowerPlex® 16 HS system.

The results indicate that selective extraction of sperm DNA is possible from mixtures in the presence of appropriate buffers. These observations were applied to different substrates and mixtures with varying ratios of sperm and epithelial cells to determine the selectivity of the extraction. The quality of the DNA recovered from pressure treatment was assessed by performing STR analysis using Promega PowerPlex® 16 HS system. Preliminary data 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.

**Differential Lysis, Pressure, Sexual Assault**

 ** Presenting Author*