A40 Differential Extraction of Mixtures in Sexual Assault Casework Using Pressure Cycling Technology (PCT)

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After attending this presentation, attendees will understand the principles of pressure cycling technology and its applicability in analyzing mixtures from sexual assault casework.

This presentation will impact the forensic science community by providing a better understanding of how this novel technology can be used to perform selective lysis of a specific type of cells and decrease the analysis time by simplifying the extraction procedure.

Forensic DNA analysis has elevated the degree of confidence in the analysis and interpretation of evidence but the bottle neck that plagues the crime labs across the country is the tedious, time consuming protocols that require practice and expertise in analyzing mixtures. 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 the 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.

Pressure cycling technology sample preparation system (PCT SPS) involves the use of pressure pulses to disrupt tissues, cells, and cellular structures enabling the recovery of their components. Barocycler® NEP2320, a commercially available instrument from Pressure Biosciences Inc, is equipped with a hydrostatic pressure chamber that generates alternating cycles of ambient and high pressure up to 45000 psi resulting in the lysis of cells. A working pressure range of 5- 45 kpsi, number of programmable cycles (1-99), duration of holding time at ambient pressure and at high pressure are the four parameters that can be controlled to achieve this objective. This can be used in conjunction with mechanical homogenization, temperature control by an external water bath, or commercially available extraction kits.

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. The cells were extracted in 1X PBS buffer (pH 7.4) with varying buffer compositions and subjected to 45000 psi pressure for 60 cycles. Samples were placed in specially designed PULSE™ tubes 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 Promega Plexor® HY system.

According to previous studies, high selectivity and improved recovery with the reducing agent, Tris (2-carboxyethyl) phosphine (TCEP) indicated the potential for highly selective detection of sperm cells in comparison to the addition of detergents or changes in temperature. These observations were applied to mixture studies of evidence obtained from various substrates such as swab and fabric. Preliminary data indicates that pressure cycling technology has application in differential extractions indicating improved extraction of sperm DNA at high pressures when compared to epithelial cells in the presence of appropriate buffers.

Pressure, Sexual Assault, Differential Extraction

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