THE OPTIMIZATION OF PRESSURE CYCLING TECHNOLOGY FOR DIFFERENTIAL EXTRACTION OF SEXUAL ASSAULT CASEWORK

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There is currently a backlog of rape kits and other forensic evidence awaiting analysis in the United States. Approximately 500,000 kits remain untested nationwide. One factor that stalls evidence processing is the extraction method itself. Differential extraction can be time-consuming, laborious, difficult to automate, and often results in poor recovery of DNA from the swab used to collect the sample A two-step protocol involving pressure cycling technology (PCT) and alkaline lysis has been devised as a rapid and selective alternative to conventional differential extraction techniques with an increased recovery of DNA. Pressure-based lysis was achieved with the Barocycler® NEP 2320 from Pressure Biosciences. The instrument includes a hydrostatic pressure chamber which is capable of applying pressures of 5-45k psi to samples which lyses cells by applying cycles of ambient and high pressure to the sample contained in a specially designed PULSE™ tube which can withstand high pressures. During the first step of the protocol, the swab containing a mixture of epithelial cells and sperm cells is subjected to pressure-based lysis in 0.4 N NaOH. The epithelial cells are larger and more diffuse which allows them to be more easily disrupted and lysed by pressure than the more compact sperm cells. After neutralization with Tris 2M (pH 7.5) and centrifugation, the sperm cells are lysed by the application of 0.4 N NaOH at 95°C for 5 minutes. After neutralization and centrifugation, the sample is ready for purification after only 20 minutes. At 1:1 or 2:1 female to male cell ratios, high selectivity and complete separation can be achieved. But at higher ratios, male allelic dropout is observed. The goal of this research is to modify and improve this protocol to generate a clean male profile even with a large excess of female epithelial cells present in the sample. Methods to accomplish this objective include extra rounds of pressure-based lysis to lyse epithelial cells left behind in the substrate, immunomagnetic cell capture to remove excess epithelial cells prior to extraction, and altering the concentration of NaOH used during pressure-based lysis for maximum recovery.