B115 Improving Recovery of Trace DNA From Cotton Swabs Using Pressure Cycling and Alkali-Based Lysis

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Learning Overview: After attending this presentation, attendees be introduced to a new application of an efficient method for lysing and extraction low amounts of epithelial cells.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing results for a method that can decrease the analysis time for trace amounts of epithelial cells. Attendees will see results of a pressure and alkali-based protocol originally designed for differential lysis optimized for the recovery of trace samples. These results provide a method to efficiently lyse and recover trace amounts epithelial cells and DNA.

Due to recent improvements in sensitivity of DNA test procedures, more and more samples consist of low level, single-source DNA and mixtures. Low levels of input DNA can result in problems with amplification, such as heterozygous peak imbalance, allelic drop out, and drop in. Much of a sample can be lost during extraction, leading to even less sample available for the amplification process.[1] DNA extraction from cotton swabs have been found to result in a 20-76% drop in recovery.[2] This loss occurs at various stages in the extraction, most commonly during wash and transfer steps. However, another major contributing factor is the irreversible adsorption of cells to the cotton matrix. This study demonstrates improved recovery of DNA using pressure cycling and alkaline lysis. This process recovers more DNA through chemical modification of cotton and pressure assisted disruption of cells.

In this study, a method for differential lysis of epithelial and sperm mixtures has been modified to improve the recovery of trace samples. Increasing temperature and modifying the amount of NaOH lead to an improved recovery of trace amounts of DNA. Cotton swabs were treated with 800µL 0.05N NaOH at 55°C for 10 cycles of 15 seconds at 20kpsi and 15 seconds at ambient pressure. This removed and lysed epithelial cells from the cotton swab in 5 minutes. The lysates are neutralized and concentrated with a DNA filter. The samples were quantified using *Alu*-based real-time PCR.

Results demonstrate high recovery of DNA from a small number of cells. Over 60% of DNA was recovered from samples containing 1,500 to 50 cells, with about 50% of DNA recovered from samples containing 20 cells. These methods were developed based off an experimental design software that used multivariate response methods to optimize the parameters of the extraction for improved recovery of DNA. Additional optimization of the technique was guided by a multivariate experimental design to discern which parameters would elicit the largest increase in DNA recovery.

Reference(s):

1. Adamowicz MS, Stasulli DM, Sobestanovich EM, Bille TW. Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. PloS one. 2014;9(12):e116351.
2. Van Oorschot R, Ballantyne KN, Mitchell RJ. Forensic trace DNA: A review. Investig genet. 2010;1(1):14.

Pressure Cycling Technology, Trace DNA, DNA Recovery