Internal developmental validation of a DNA differential extraction protocol for forensic application

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Sexual assault samples are generally composed of a mixture of epithelial cells and spermatozoa from both the victim and suspect. The separation of this mixture is necessary to distinguish single-source DNA profiles which are easier to interpret and yield greater statistical significance. Several analytical methods have been established for the differential extraction of DNA from epithelial and sperm cells; however, they may be time-consuming, require several tube transfers increasing the risk for contamination and/or sample loss, and are often difficult to automate. Issues may also arise during cell-type separation as the wash steps incorporated may cause loss of sperm, reducing the amount of DNA recovered. This loss of biological material could mean the difference between generating a DNA profile or not in samples with low levels of semen.

This study aimed to develop and validate an in-house differential extraction protocol which would reduce cell loss by finding a buffer which creates a barrier to protect the pellet. Various density liquids were assessed including diethyl glutarate, dimethyl glutarate, and 1-chloro-2-methyl-2-propanol; selection was based on how effectively the fractions were separated while ensuring that no inhibition was detected. Mock sexual assault samples were created following three different dilution series and STR profiles were developed following five different extraction methods. Wash steps were saved and viewed microscopically to assess spermatozoa loss and effectiveness of the selected buffer.

PCIA, Qiagen EZ1® with barrier buffer, and modified PCIA with barrier buffer extraction methods show a significantly higher recovery of DNA than Promega's Differex™ System and DNA IQ™ with barrier buffer within the epithelial fractions. Within the spermatozoa fraction, DNA IQ™ with barrier buffer showed a significantly better yield than all other extraction methods, followed by PCIA in the 1:1 dilution, and no significant differences found in 1:10 or 1:100. Final determination on the preferred method was based on purity of the spermatozoa cell counts from the reserved wash steps. Slide searches show PCIA without use of a barrier buffer having the least amount of cell loss.