**Introduction**

Forensic scientists must handle a significant workload of sexual assault kits. Possibly the most work-intensive part of processing the kits is the extraction of DNA from stains found on items of clothing or swabs. In particular, differential separation, the process of separating epithelial cells from sperm cells, is an especially lengthy process. Since most validated methods are currently performed manually, which subjects analysts to hours of repetitive motion, it is worthwhile to investigate the potential of instruments like the QIAcube®. This instrument was originally designed for cell lysis and the extraction of nucleic acids and proteins, and in this study, we investigated its potential to perform differential separations.

**Methods**

**Sample Preparation**

For the cross-contamination study, female blood was mixed with semen. For the sensitivity, reproducibility, and matrix studies, a 1:3 serial dilution series of semen was prepared with phosphate buffered saline (PBS) (pH 7.2). For mixtures, an approximate 2:1 saliva dilution with PBS was prepared and mixed with the semen dilution.

**Automated Separation**

An epithelial cell lysis "master mix" (94.8% Buffer G2, 4.7% Proteinase K, and 0.5% carrier RNA) was added, followed by an incubation for 3.5 hours at 65°C. The QIAcube® performed the differential separation and washing of the sperm pellet, then added a sperm lysis "master mix" (95.8% Buffer G2, 4.7% Proteinase K, 19.9% DTT, and 0.5% carrier RNA). Sperm fractions were incubated on a thermomixer at 70°C for 10 minutes at 900 rpm to complete lysis. DNA purification was performed on the EZ1® Advanced XL.

**Manual Separation**

An epithelial cell lysis "master mix" (95.2-96.2% Buffer G2 and 4.0-4.8% Proteinase K) was added, followed by an incubation for 1.5 hours at 65°C. Samples were centrifuged at 13,200 rpm for 5 minutes, the epithelial fraction was separated out manually, 1 μL carrier RNA was added to it, and the sperm pellet was washed at least three times with 500 μL Buffer G2. The sperm fraction received a lysis "master mix" (97.8% Buffer G2, 4.7% Proteinase K, 19.9% DTT, and 0.5% carrier RNA), then incubated on a thermomixer at 70°C for 10 minutes at 900 rpm. DNA purification was performed on the EZ1® Advanced XL.

For the cross-contamination study, samples containing the biological mixture were placed alternately between "blanks" containing Buffer G2 and Proteinase K in the centrifuge. Positions were switched for the second run. The sensitivity study used two replicates of semen only and two replicates of semen with saliva to each method (automated and manual). The reproducibility study used the highest concentration of semen dilution with saliva, and both methods used 18 replicates. The matrix study used the lowest semen dilution with saliva, added to duplicates of matrices: towel, jeans, white t-shirt, brown t-shirt, blue sock, and swab.

**Results**

**Sensitivities**

![Average Human Quantitation Values](image)

**Reproducibility**

![Quantitation of sperm fractions: manual versus automated methods](image)

Matrix Dropout was seen in the matrix study that was not seen in liquid studies. Dropout was not consistent within matrix type. Questions regarding the matrix study are currently being addressed.

**Conclusions**

Samples differentially extracted using the QIAcube® yielded similar sensitivity compared to the manual method (Figures 1 and 2).

Samples differentially extracted using the QIAcube® yielded more reproducible results compared to a group of six experienced analysts performing the manual method (Figures 3 and 4). While the analysts consistently provide work of high quality, the instrument studied was able to extract less variable amount of DNA from sperm and epithelial cells.

Matrix study requires more research, which is the focus of current research being performed at Marshall University.

The instrument has a high initial cost, but time spent in use and performing several runs per day will even out expenses.

Easy on the analyst: streamlines workflow, requires less repetitive motion, very easy to learn.

**References**

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