Comparison of the QIAcube® to Manual Differential Separation: Man Versus Machine

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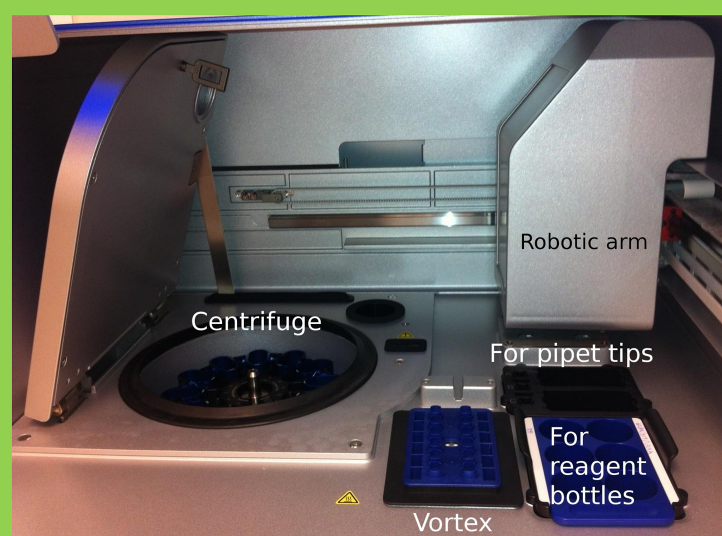

Abstract

Sexual assault is a serious public safety concern worldwide, with the resulting caseload backlog posing significant challenges for forensic laboratories. In an effort to identify ways to reduce these backlogs and benefit a scientist's workflow, it is worth evaluating the use of automation. This study focused on determining the utility of the QIAGEN® (Hilden, Germany) QIAcube® for differential separation of samples, and compared it to the current manual method. This study evaluated the QIAcube® using a custom protocol to perform differential separations on up to 12 mock sexual assault samples at a time. Experiments included a cross-contamination study, a sensitivity study, a reproducibility study, a matrix or mock evidence study, and a cost analysis. All studies were performed by a novice student using the QIAcube®. For comparison, the sensitivity and reproducibility studies were also performed by one or more experienced analysts. The QIAcube® proved to be a very efficient way to perform differential separations, with excellent sensitivity and reproducibility and no sign of cross-contamination between samples. Conversely, there was some reagent waste with the automated method. The factor of general human error – for example, bumping a tube and having to re-pellet sperm cells – is eliminated with automation. In conclusion, the use of the QIAcube® has the potential to help a scientist work more efficiently simply by freeing an analyst or technician from repetitious pipetting and centrifuging, and by helping to streamline an analyst's workflow. *(Photographs of the QIAcube® by Josh Stewart, MSFS.)*

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 1:2 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 1.5-2 hours at 56°C. The QIAcube® performed the differential separation and washing of the sperm pellet, then added a sperm lysis "master mix" (75.8% Buffer G2, 4.7% Proteinase K, 19.0% 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 1-2 hours at 56°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" (75.8% Buffer G2, 4.7% Proteinase K, 19.0% 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 (automatic 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

Cross-contamination: no evidence of cross-contamination.

Sensitivity:

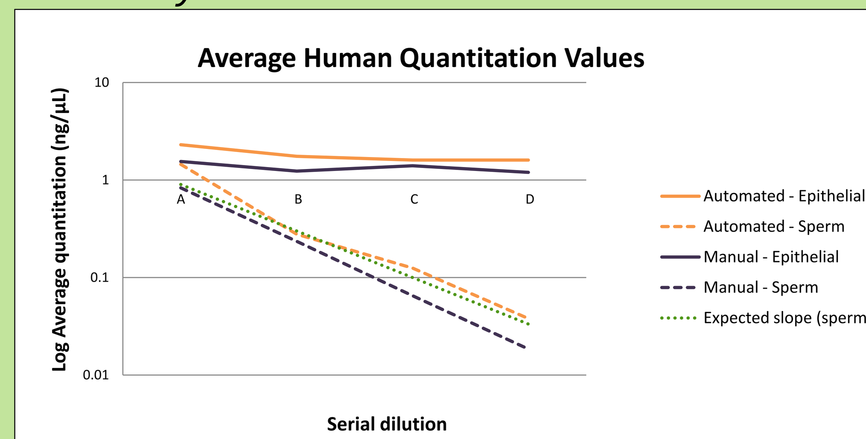

Figure 1: Quantitation values of the sensitivity study (semen with saliva). Both the manual and automated extraction methods followed the expected decreasing trend.

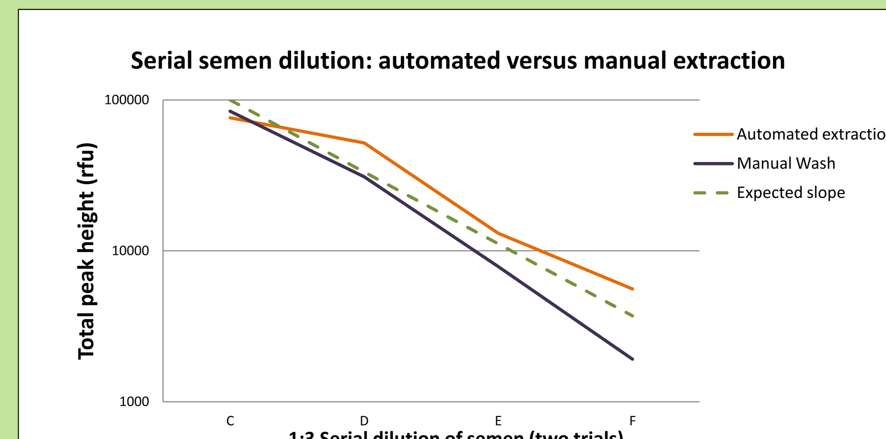

Figure 2: Total peak height sum of two trials per test (manual and automated) after capillary electrophoresis. Dilutions A and B are not shown because they were normalized before amplification.

Reproducibility:

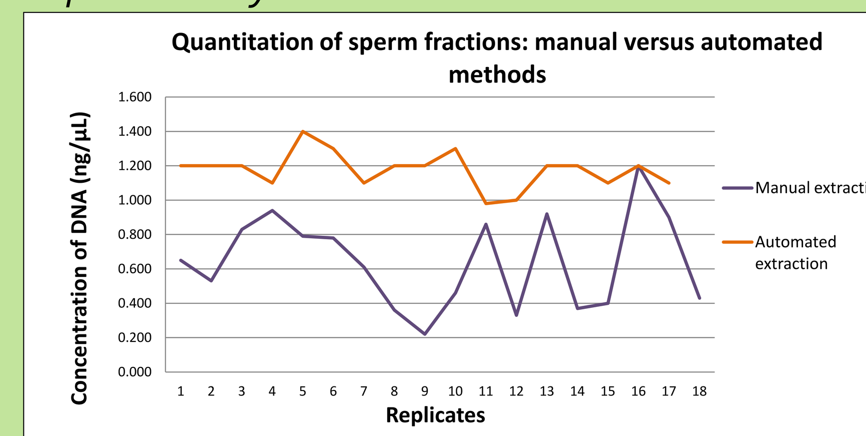

Figure 3: Quantitation values of the sperm fractions for the reproducibility study. CV for manual extraction was 43%; CV for automated extraction was 9%.

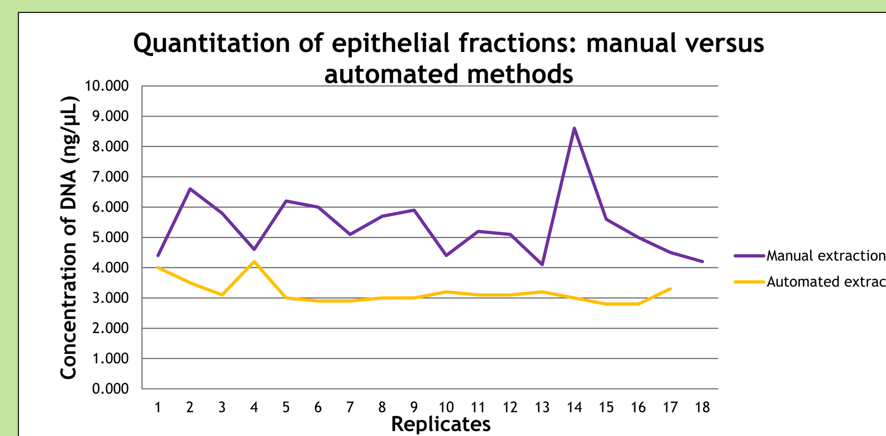

Figure 4: Quantitation values of the epithelial fractions for the reproducibility study. CV for manual extraction was 20%; CV for automated extraction was 12%.

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.

Cost Analysis:

Table 1: QIAcube® Set-Up and EZ1® Purification Cost

Item	Amount per set-up	Number needed	Total
EZ1® expense (cartridge, G2, Pro K, etc.)	8.104166667	2	16.20833
Tips - 1000uL QIAGEN®, wide-bore	0.077734375	10	0.777344
Rotor Adapter and Elution Tube	0.150833333	1	0.150833
Surplus Proteinase K	0.0267	140	3.738
DTT (35000uL)	0.003371429	640	2.157714
Buffer G2 (260mL) - 6900uL surplus needed	1.756846154	1	1.756846
Total for e-cell & sperm cell	-	-	**24.78907**

Per Season Seferyn, MSFS

- Instrument cost: $17,802
- $17.78 per sample using the QIAcube® (after initial set-up)
- Manual method costs $18.57 per sample

Per Season Seferyn.

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 a 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.

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