Evaluation of manual differential separation in comparison to QIAcube® automation

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Abstract

The QIAGEN® QIAcube® is a robotic workstation capable of automating separation of sperm and epithelial cells for up to 12 samples via a customized protocol. This offers the potential to streamline the analysis of sexual assault samples by improving analyst efficiency and eliminating the potential for human error. An internal validation was performed at the Phoenix Police Department (PPD) Crime Laboratory according to the Scientific Working Group on DNA Analysis Methods (SWGDAM) and the FBI Quality Assurance Standards (QAS) validation guidelines. This study assessed the possibility of using the QIAcube® for separating sperm and epithelial cells by comparing it to the manual method currently validated and in use at the PPD Crime Lab. Experiments included a buffer study and studies of sensitivity and linearity, reproducibility, and cross-contamination. The QIAcube® demonstrated sensitivity and reproducibility comparable to the current manual method and showed no cross-contamination.

Introduction

There were 1,725 rapes reported in Arizona in 2012, and there has been an increasing rate in the number of rapes that occur annually in the state (1, 2). As a result, there has been an increase in the number of sexual assault cases to be analyzed, which leads to a backlog of evidence to be processed. The QIAcube® is a robotic workstation designed to automate the purification of proteins and nucleic acids, however custom protocols can be obtained to use the QIAcube® for other procedures, such as the separation of sperm and epithelial cells (3). Efficacy and reliability of the QIAcube® using the custom protocols was compared to that of the currently validated manual method in use at the PPD Crime Lab. The currently validated manual method requires many steps and a lot of time to complete. This study focused on comparing the manual wash portion of differential extraction to the automated version on the QIAcube®.

Materials

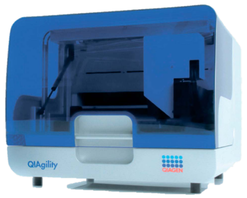

Figure 1 The Qiagen® QIAcube® (4)

Figure 2 The Qiagen® QIAcube®: inside view (5)

- **Extraction:** Qiagen® QIAcube, Qiagen® QIAamp® DNA Investigator Kit, Qiagen® QIAsymphony, Qiagen® QIAsymphony® DNA Investigator Kit
- **Quantitation:** Promega™ Plexor® HY System, AB® 7500 Real-Time PCR System
- **Amplification:** AB® AmpFℓSTR® Identifiler® Plus kit, AB® 9700
- **CE and Analysis:** AB® 3500xL Genetic Analyzer, AB® GeneMapper® ID-X Software v1.4

Methods

Sample Preparation

Sperm ratings, based on sperm searches, were given to semen dilutions and stains made with semen dilutions. Epithelial cells were digested using an epithelial cell lysis mixture containing 95% Buffer ATL and 5% Proteinase K. The sperm fractions processed by the QIAcube® were washed four times, and a sperm lysis buffer containing 75% Buffer ATL, 20% DTT, and 5% Proteinase K was added. The sperm fractions processed manually were washed three times, and a sperm lysis buffer containing 80% Buffer ATL, 10% DTT, and 10% Proteinase K was added.

Buffer study

An initial study was run on the QIAcube® comparing Buffer ATL and Buffer G2. A 1:4 semen dilution series was prepared, six replicates were prepared for differential extraction, and sperm ratings ranging from negative to 4+ were assigned to each dilution by a trained analyst. 20 µL of each dilution was spotted onto a cotton t-shirt.

Sensitivity and linearity study

A 1:2 semen dilution series was prepared, and six 50 µL replicates were prepared for differential extraction.

Table 1 Semen dilution series

Dilution	Volume of semen dilution	Volume of TE^{-4} buffer	Calculated human [DNA] (ng/ µL) in 50 µL eluate
A	300 µL	0 µL	4.93
B	100 µL of A	200 µL	1.64
C	100 µL of B	200 µL	.548
D	100 µL of C	200 µL	.183
E	100 µL of D	200 µL	.0609
F	100 µL of E	200 µL	.0203

Reproducibility study

Samples were prepared by using dilutions C – E since the quantitation values and sperm ratings for these dilutions were most representative of the casework samples typically received for DNA analysis at the PPD Crime Lab. Samples were prepared by adding 50 µL of the 1:2 saliva dilution to 50 µL of dilutions C – E. One set of samples consisted of dilutions C – E in duplicate, with the first duplicate being mixed with female saliva and the second duplicate being mixed with male saliva.

Cross-contamination study

The risk of cross-contamination between samples was assessed using the same sample sets used in the reproducibility study, with the two studies being run simultaneously. Samples from the reproducibility study were loaded into the QIAcube® with reagent blanks in an alternating pattern

Results

Buffer study

Similar profiles were obtained from samples processed with Buffer ATL and from samples processed with Buffer G2. Buffer ATL was used for the remaining studies.

Sensitivity and linearity study

Full profiles from sperm fractions were obtained for dilutions A through E, while dropout occurred at dilution F for the manual method and the first QIAcube®. Dropout occurred at dilution E for the second QIAcube®. Full profiles for epithelial fractions were obtained for dilutions A – D, with dropout occurring at dilution E, for all samples. Overall, results were consistent between the automated and manual separation methods.

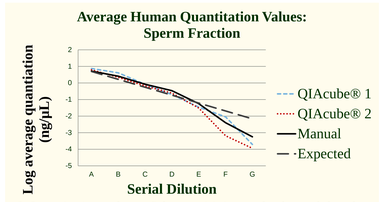

Figure 3 Quantitation values of the sensitivity study showed that the sperm fractions of the automated and manual extraction methods showed a decreasing trend as expected, and that results were consistent between methods.

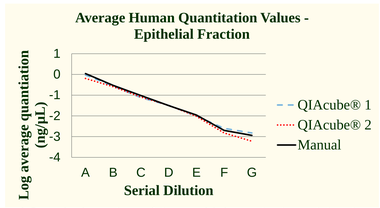

Figure 4 Quantitation values of the sensitivity study showed that the sperm fractions of the automated and manual extraction methods showed a decreasing trend as expected, and that results were consistent between methods.

Reproducibility study

The expected profiles were obtained from samples processed manually and samples processed on the QIAcube®. Quantitation values of samples processed on the QIAcube® were similar to those of samples processed manually.

Cross-contamination study

The expected profiles were seen both for samples sets processed manually and sample sets processed on the QIAcube®. Reagent blanks run in both even and odd positions on each QIAcube® yielded a clean profile with no peaks, and all 48 samples were free of contamination.

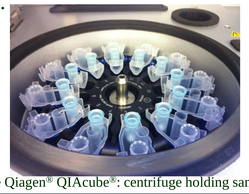

Figure 5 The Qiagen® QIAcube®: centrifuge holding samples (6)

Discussion

Further studies on the ability of QIAcube® to recover sperm cells from a matrix would be worth performing. It would also be worthwhile to consider further customization of the procedure to have the analyst add the sperm lysis buffer instead of the QIAcube® since the final volume of the sperm fractions has to be brought up before being placed on the QIAsymphony®. Additionally, the PPD plans to evaluate a material modification to use the same sperm lysis buffer ratios as the current PPD method since the QIAcube® uses a much higher concentration of DTT per sample.

Conclusion

The QIAcube® has the potential to standardize the differential wash procedure while giving analysts free time to take care of other tasks while the instrument runs. Due to the extra amount of processing that must be done to samples, the QIAcube® did not prove to be a faster method for the separation of sperm and epithelial cells, however it does reduce the potential for human error, and it did prove to provide results with equal sensitivity and reproducibility to manual processing. More studies must be performed to determine if the QIAcube® can fit in with the current workflow of the PPD Crime Lab.

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