Comparison of QIAcube® Differential to Manual Differential Extraction When Purified Using the QIAamp® DNA Blood Mini Kit

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Abstract

In a 2008-2012 survey performed by the U.S. Department of Justice, an average of 237,868 victims reported being sexually assaulted each year, which calculates to an occurrence roughly every two minutes. Although only about half of all sexual assaults are reported, a great deal of time and effort goes into processing evidence from these cases due to the potential of samples containing female-male mixtures on which differential extractions must be performed. This study focused on determining the utility of Qiagen®'s (Hilden, Germany) QIAcube® for differential extraction of samples and compared it to the Qiagen® QIAamp® DNA Blood Mini manual method currently being used by the Washoe County Sheriff's Office Crime Lab (WCSO). This study evaluated the QIAcube®'s abilities, using a standard protocol, to perform differential separations on up to 12 mock sexual assault samples at a time. Experiments included a buffer study comparing three potential buffers incorporated into the lysis mixtures; a temperature study comparing two different sperm lyse temperature protocols; a sensitivity study based on a 1:3 semen dilution series, with and without female epithelial cells present; a cross contamination study using mixed female blood and semen; as well as a matrix and mock evidence study consisting of a mixture of female epithelial cells and semen pipetted onto different substrates along with various proficiency test samples. All studies were performed by a graduate student using a combination of four QIAcubes®. For comparison, the sensitivity and reproducibility studies were also performed by an experienced analyst. There was no sign of cross-contamination between samples, even though the tubes remain open all at once in the instrument. Interestingly, the manual method consistently yielded DNA concentrations approximately twice as high as the QIAcube® for the sperm fraction. Extensive troubleshooting was performed to include the use of different reagents and temperatures as well as a variety of protocol variations. In conclusion, the WCSO Crime Lab will not be utilizing the QIAcube® to perform differential extractions unless future modifications of the standard protocols result in higher male yields.

Introduction

Forensic scientists must handle a significant number of sexual assault kits, resulting in backlogged evidence requiring extensive analytical processing. The most pertinent evidence collected in these cases contains both the DNA from the victim and perpetrator, requiring a special technique called differential extraction. Differential extractions incorporate a combination of phase separation with differential centrifugation to isolate sperm cells from other cell types in order to generate two distinct DNA profiles. Traditionally, differential extractions have been performed manually, requiring an analyst to undergo repeated pipetting and multiple centrifugation steps. Due to the hands-on nature of the approach the quality and consistency of the separations tend to be variable from analyst to analyst. Bringing an automated differential extraction procedure online would benefit analysts by not only reducing the backlog of the laboratory but also by streamlining the workflow of a lengthy process and removing analyst to analyst variability.

Methods

Sample Preparation

For the buffer study, temperature study and sensitivity studies, a 1:3 serial dilution series was prepared with TE^{-4} solution. For the cross–contamination study, female blood was mixed with semen. For the substrate study, a mixture of neat semen was mixed with female saliva.

Automated Separation

The samples were vortexted for 10 seconds with a mixture of 500 µL of a digest buffer and 15 µL of Proteinase K. The samples were then incubated at 56 °C for 1 hour at 550 rpm on a thermomixer. After incubation the samples were again vortexed and transferred to the appropriate 1.5 mL elution tube placed in the collection tube position of a rotor adapter. The rotor adapters were placed in the centrifuge and empty 2 mL elution tubes were placed in the shaker of the QIAcube®. The first protocol was on the QIAcube® for centrifugation of the samples and separation of the epithelial fraction from the samples. This protocol also performs two sperm washes. After the first protocol was complete, the epithelial fractions were removed, capped and stored until further processing could be done. The second protocol was selected on the QIAcube® for two additional sperm washes and the addition of the sperm lysis buffer that included a digest buffer, Proteinase K and DTT. Once the second protocol was finished the sperm fractions were vortexed for 10 seconds and incubated at 70 °C for 10 minutes at 900 rpm or at 56 °C for 1 hour at 550 rpm. The epithelial and sperm fractions were further purified using the QIAamp® DNA Blood Mini Kit on the QIAcube®.

Manual Separation

Manual samples followed the QIAamp® DNA Blood Mini Kit manual procedure currently validated in the WCSO Crime Lab. 500 µL of the in-house digest buffer along with 15 µL of Proteinase K were added to the samples and incubated at 56 °C for 1 hour at 550 rpm on a thermomixer. After incubation, the samples were centrifuged for 5 minutes at 15,000 on an Eppendorf® (Hauppauge, NY) Centrifuge 5424. All but approximately 50 µL of the resulting supernatant was removed and transferred to a new tube as the epithelial fraction. Between 500 and 1,000 µL of digest buffer was added to suspend the sperm pellet, vortexed vigorously and again the tube was centrifuged at maximum speed for 5 minutes. All but 50 µL of the supernatant was discarded. The wash step was repeated a minimum of 3 times and a maximum of 5 times before the sperm were lysed. The sperm lysis buffer, consisting of 500 µL of digest buffer, 15 µL Proteinase K and 20 µL of DTT, was added to the samples and incubated at 56 °C for at least 1 hour and no longer than 24 hours at 550 rpm .The sperm and epithelial fractions were purified manually using the QIAamp® DNA Blood Mini Kit.

Results

Cross-contamination Study: No evidence of cross contamination detected between the samples within the QIAcube® instrument.

Buffer Study:

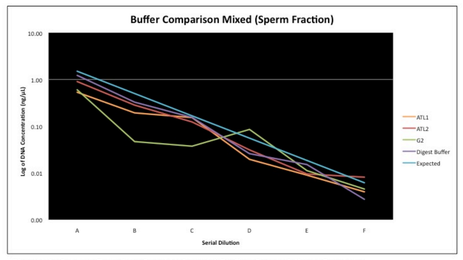

Figure 1: Sperm Fraction Comparison of Buffer Study Mixed Samples.

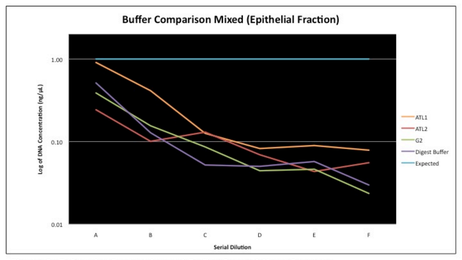

Figure 2: Epithelial Comparison of Buffer Study Mixed Samples

The WSCO Crime Lab in-house digest buffer was determined to be the optimal buffer to use for the differential extraction procedure. The epithelial fraction shows decreasing values similarly seen in the sperm fractions. The decreasing trend may be due the decreasing amount of epithelial cells contributed by the semen sample and varying amounts of cells contained in the female sample, however, studies were not performed to verify this possibility.

Temperature Study:

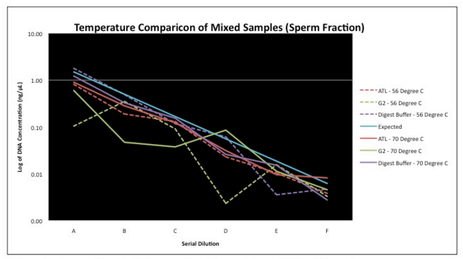

Figure 3: Sperm Fraction Temperature Comparison of Mixed Samples

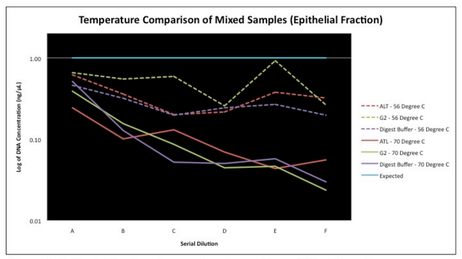

Figure 4: Epithelial Fraction Temperature Comparison of Mixed Samples

Comparing the two temperature protocols between each buffer option, it was determined that either protocol could be used. The cross-contamination study and substrate study were performed using the WCSO Crime Lab in-house buffer and the 70 °C for 10 minutes at 900 rpm in the interest of saving time.

Sensitivity Study:

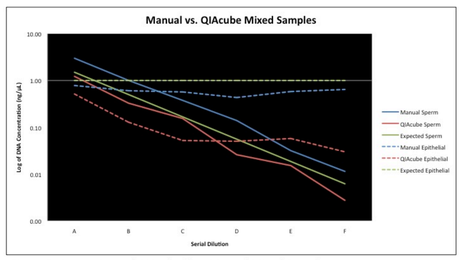

Figure 5: Comparison of QIAcube® to Manual Mixed Samples

The manual method was able to extract more DNA than the QIAcube® when performing differential extractions. The expected amounts of DNA are inaccurate because they were determined using the QIAcube® method when they should have been determined using the manual method, since it was the current validated method for differential extractions.

Substrate Study: Samples exhibited varying amount of inhibition depending on the substrates they were placed on. White cotton cloth and cotton swabs extracted the most DNA from the five samples tested (white cotton, colored cotton, buccal swabs, jeans, and leather). The leather and jean substrates showed inhibition from the presence of tannic acid and indigo dye, respectively.

Conclusions

The QIAcube® performed differential extraction without cross-contamination of adjacent samples. The substrates most likely to produce a DNA profile from the sperm fraction are cotton swabs and white cotton. Results showed that the optimal buffer to use on the QIAcube® as an epithelial lysis buffer, wash buffer and sperm lysis buffer was the in-house lysis buffer of the WCSO Crime Lab. It was determined that the difference between the 70 °C incubation for 10 minute at 900 rpm and the 56 °C incubation for 1 hour at 550 rpm was not significant enough to choose one over the other. The results from the sensitivity study show the manual method using the QIAamp® DNA Blood Mini Kit far out performs the automated method. The WCSO Crime Lab has determined that the QIAcube® differential wash protocol will not be utilized for differential extractions unless future modifications of the standard protocols result in higher male yields. For the present time the lab will continue using the QIAamp® DNA Blood Mini Kit manually for differential extractions.

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