Application Note

Improved differential cell lysis and extraction using an automated protocol on the QIAcube® Instrument

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Introduction

To accurately perform STR assays for human identification in sexual assault casework, sperm cells and epithelial cells must be separated using a differential lysis protocol. Although traditional differential lysis protocols using organic extractions are very effective in separating these two cell fractions, they are typically highly labor intensive, and their efficiency is dependent on an analyst's skill level. This can lead to a lack of reproducibility in sample preparation, inefficient cell separation and delayed processing, contributing to a backlog in cases.

New, automated methods using robotic systems offer a solution to these technical hurdles. In particular, QIAGEN's QIAcube Instrument is equipped with a protocol that combines cell lysis, separation of sperm and non-sperm cell fractions and DNA purification, where the only manual steps involve loading plasticware, reagents and samples into the instrument. In this application note, we compare the efficacy of the differential lysis and extraction protocol automated on the QIAcube Instrument with two commonly used manual methods. We assessed the success of these approaches using qPCR-based DNA quantification and STR amplification, followed by analysis with capillary electrophoresis.

Materials and methods

Samples

Mock sexual assault samples were created. Epithelial cells were obtained from buccal swabs of 11 female volunteers, while sperm cells were obtained from semen samples from 4 male volunteers. Buccal swabs for reference DNA samples were also taken from the male volunteers. The mock sexual assault samples were produced using 3 µl neat semen from a single volunteer and combined with a dried buccal swab from a female volunteer.

Cell lysis and DNA extraction

Mock samples were extracted using one of three methods: An automated QIAcube protocol, QIAGEN's manual differential extraction method or a standard organic differential extraction method.

Automated QIAcube protocol: A QIAamp® DNA Investigator Kit (QIAGEN) [1] was used in combination with the QIAcube Instrument (QIAGEN) following the manufacturer's instructions [2]. Epithelial cells were lysed using the "Buccal swab spin protocol part A (lysis)" program, followed by separation and sperm cell lysis using the "Differential wash protocol" program. A from ▷

both fractions was then purified using the "Buccal swab spin protocol part B (purification) program" with elution volumes of 60 µl for the two fractions. QIAGEN manual differential extraction: Cells from the swabs were lysed in stain extraction buffer (1 mol/l Tris-HCl, ddH_2O, 5 mol/l NaCl, 0.5 mol/l EDTA, 10% SDS, pH = 8.0) and proteinase K (20 mg/ml). Lysates were collected using DNA IQ™ spin baskets, after which the epithelial fraction (supernatant) was separated from the sperm pellet. Following sperm lysis, both fractions were purified manually using the QIAamp DNA Investigator Kit, according to the manufacturer's instructions [1].

Reference DNA profiles for each volunteer were produced using a QIAamp DNA Blood Mini Kit (QIAGEN) on the QIAcube Instrument [3].

STR amplification and analysis

Total human and human male DNA was quantified in the DNA extracts using the Investigator® Quantiplex® HYres Kit (QIAGEN) on an ABI PRISM® 7500 Sequence Detection System (Life Technologies™). DNA samples with the highest, lowest and median human:male DNA ratios were used for STR amplification using the AmpFℓSTR® Identifiler® PCR Amplification Kit (Life Technologies) with a GeneAmp® 9600 PCR System (PerkinElmer Incorporation).

Results and discussion

We first examined the DNA yields for the sperm and non-sperm cell fractions produced using the automated QIAcube, manual QIAGEN and manual organic differential lysis and extraction methods. As shown in Figure 1A, the automated QIAcube method produced a slightly higher DNA yield for the non-sperm fraction compared to the manual QIAGEN method, although this difference was not statistically significant. The manual organic method produced higher yields of non-sperm fraction DNA than either of the other two methods. The three methods produced similar total yields of sperm fraction DNA, with no statistical differences between them (Figure 1B). Importantly, all three methods produced greater than 75 ng of sperm fraction DNA, which is sufficient for downstream STR amplification and profile creation.

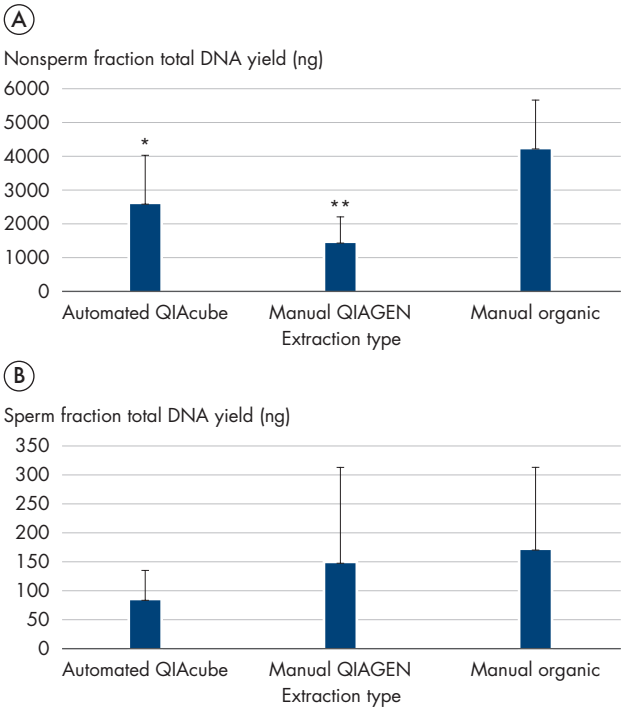

Figure 1. DNA yields from automated and manual differential lysis and extraction methods. A The automated QIAcube method produced a non-sperm DNA yield that was not significantly different than the manual QIAGEN method. The manual organic method produced a higher yield that the automated QIAcube or manual QIAGEN methods. **B** The sperm DNA yield was not significantly different between the three methods. n = 11 for automated QIAcube and manual QIAGEN, n = 10 for manual organic.

Next, we evaluated the ratio of total human DNA to male DNA in the sperm fractions isolated using each method. For optimal STR profile development, the ideal ratio of human:male DNA is 1:1; ratios greater than this reflect a high female contribution and can result in mixed STR profiles, which are more difficult to interpret. The automated QIAcube and manual organic methods,

with ratios of 0.94:1 and 1.04:1, respectively, performed notably better than the manual QIAGEN method, with a mean ratio of 1.37:1 (Figure 2). A human:male DNA ratio greater than 1.2:1 reflects a substantial female contribution. While 72% of samples isolated using the manual QIAGEN and 30% of samples isolated with the manual organic method exhibited ratios of >1.2:1, the automated QIAcube method produced no sperm fraction samples with a human:male DNA ratio greater than 1.2:1 (Figure 2). These results indicate that of the three methods, the automated QIAcube approach was most successful in separating the sperm from the non-sperm fraction.

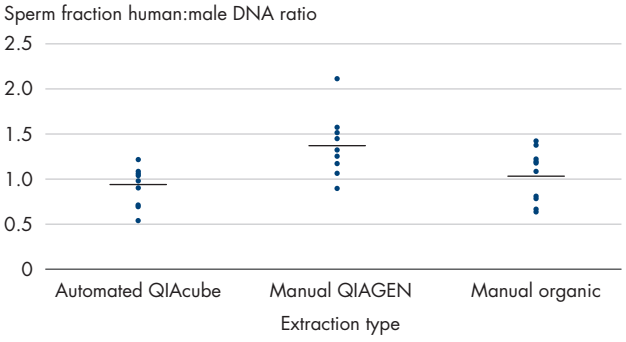

Figure 2. Ratio of human:human male DNA in the sperm fractions produced using the automated and manual differential lysis and extraction methods. The automated QIAcube method produced a sperm cell fraction with the lowest female contribution compared to the two manual methods. n = 11 for automated QIAcube, n = 10 for manual QIAGEN and manual organic.

Finally, we performed STR amplifications using the isolated sperm fraction DNA and developed STR profiles for each sample. Consistent with the human:male DNA ratio assessment, the automated QIAcube method produced mixed STR profiles for only 2 of the 6 samples, while both manual methods resulted in mixed STR profiles for 5 of 6 samples (Table 1). In addition, the median number of female alleles detected in each sperm fraction was 6–8x higher when the manual methods were used compared to the automated QIAcube approach (Table 1, Figure 3).

Table 1. STR profile data from the purified sperm fraction DNA

Method	Number of mixtures detected	Median number of female STR alleles
Automated QIAcube	2/6	1.5
Manual QIAGEN	5/6	12
Manual organic	5/6	9

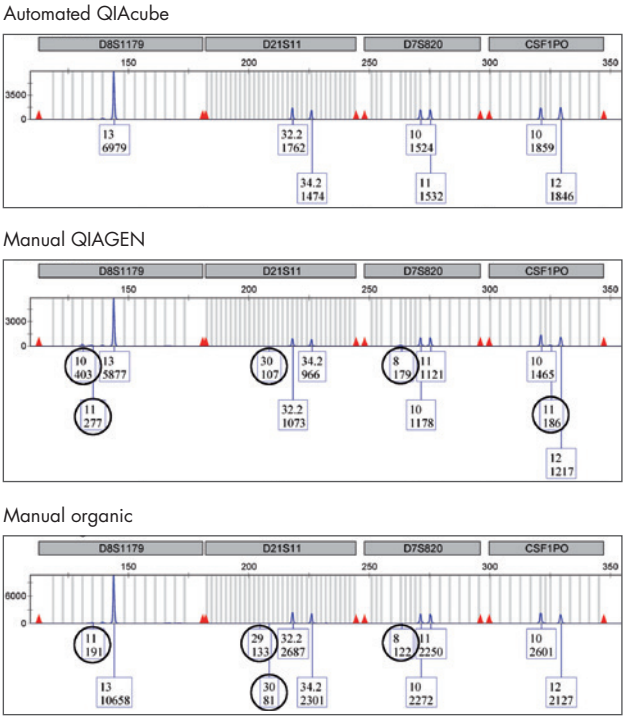

Figure 3. Example STR profile electropherograms for the sperm fractions isolated using the automated and manual differential lysis and extraction methods. The automated QIAcube method resulted in a single-source STR profile for this representative sample, while the two manual methods exhibited evidence of female donor alleles (circles).

Conclusion

Taken together, these results indicate that automated differential cell lysis and extraction on the QIAcube Instrument using mock semen-containing assault samples was superior to the two manual methods assessed in this study. The automated QIAcube approach yielded similar amounts of total DNA as the manual methods, but was more effective in separating sperm from non-sperm cells. This enabled a greater number of ▷

single-source STR profiles to be produced and minimized the contribution of female DNA.

Summary

The QIAcube Instrument allows forensic analysts to perform fully automated differential cell lysis and DNA extraction of semen-containing sexual assault samples.

Compared to manual approaches, the QIAcube method provides:

- Faster, more efficient protocol that reduces hands-on time by at least 90 minutes
- Better separation of sperm and non-sperm fractions, reducing the contribution of female DNA to downstream analyses
- Improved STR profile quality, with a reduction in mixed STR profiles and subsequent interpretation time

References

1. *QIAamp DNA Investigator Handbook*. 5th ed. January 2020.
2. *QIAcube User Manual*. Version 1.3. March 2018.
3. *QIAamp DNA Mini and Blood Mini Handbook*. 5th ed. May 2016.
4. Goldstein, M. C., Cox, J. O., Seman, L. B. and Cruz, T. D. (2020) Improved resolution of mixed STR profiles using a fully automated differential cell lysis/DNA extraction method. *Forensic Sci. Res.* **5**, 106–112.

Ordering Information

Product	Contents	Cat. no.
QIAcube Connect	Instrument, 1-year warranty on parts and labor	9002864
QIAcube HID Differential Washing Station	Differential Wash Protocol Pack, QIAcube (110V), Starter Pack, Installation, IQ/OQ Services, Guided Validation Support (offered only in certain areas; please contact your QIAGEN representative)	9002160
QIAamp DNA Investigator Kit (50)	For 50 DNA preps: 50 QIAamp MinElute® Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56504
QIAamp DNA Blood Mini Kit (50)*	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
Investigator Quantiplex HYres Kit (200)	Reaction Mix FQ, Primer Mix IC YQ, Control DNA Z1, QuantiTect® Nucleic Acid Dilution Buffer	387116

*Larger kit sizes are available; see **www.qiagen.com** for details.

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