Internal Validation of the PTC Erase Sperm Isolation Kit

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Abstract

Sexual assault evidence frequently consists of a biological mixture of male and female cellular material. When processing these samples, an often-time-consuming and laborious differential extraction is performed in an attempt to separate the male sperm DNA from the female epithelial cell DNA. Failure to separate these cell types may result in a DNA mixture, which can make interpretation and statistical evaluation difficult and time-consuming. Therefore, one of the most important steps in the differential methodology are the sperm cell pellet washes which are performed to remove excess epithelial cell DNA. The Erase Sperm Isolation Kit is a differential extraction kit that utilizes nuclease activity instead of sperm cell pellet washes to destroy epithelial cell DNA, leaving the sperm cells unaffected and intact, ideally resulting in a single source male DNA profile. Overall, the results of this validation demonstrate that the Erase Sperm Isolation Kit produces comparable results to the differential separation method currently utilized by the Palm Beach County Sheriff’s Office (PBSO). If implemented, the main advantage of the Erase Sperm Isolation Kit protocol would be the reduction of sample handling and processing steps.

Introduction

Sexual assault samples are commonly submitted and often contain a biological mixture of male and female cellular material [2]. The extraction step of the DNA workflow has the most potential for sample loss and contamination, with the quality of a DNA extraction process directly affecting the recovery of DNA typing results from samples [4]. PBSO currently employs the QIAEN® E21™ DNA Investigator® Kit in conjunction with the QIAEN® E21™ Advanced XL for this extraction. This methodology utilizes an automated purification step, but still requires the use of manual sample pre-processing steps. In order to minimize the potential for sample loss and/or contamination, it is important to eliminate as many manual intervention steps as possible while still maintaining optimum DNA recovery. For those reasons, the Paternity Testing Corporation (PTC) Erase Sperm Isolation Kit is a possible alternative to the current differential sample pre-processing protocol. An internal validation was performed at PBSO to evaluate if the Erase Sperm Isolation Kit would provide a more efficient method for sample pre-processing resulting in a quality and yield of DNA that was equal or superior to the current method.

Materials and Methods

Samples

Sexual assault samples were commonly submitted and often contain a biological mixture of male and female cellular material [2]. The extraction step of the DNA workflow has the most potential for sample loss and contamination, with the quality of a DNA extraction process directly affecting the recovery of DNA typing results from samples [4]. PBSO currently employs the QIAEN® E21™ DNA Investigator® Kit in conjunction with the QIAEN® E21™ Advanced XL for this extraction. This methodology utilizes an automated purification step, but still requires the use of manual sample pre-processing steps. In order to minimize the potential for sample loss and/or contamination, it is important to eliminate as many manual intervention steps as possible while still maintaining optimum DNA recovery. For those reasons, the Paternity Testing Corporation (PTC) Erase Sperm Isolation Kit is a possible alternative to the current differential sample pre-processing protocol. An internal validation was performed at PBSO to evaluate if the Erase Sperm Isolation Kit would provide a more efficient method for sample pre-processing resulting in a quality and yield of DNA that was equal or superior to the current method.

Results

Sensitivity-Stochastic/Mixture Study

Table 1: Summary of sensitivity-stochastic/mixture study results for the sperm fraction separated using both the PTC Erase Sperm Isolation Kit and PBSO’s differential separation protocol

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Erase Differential Separation</th>
<th>PBSO Differential Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Blood</td>
<td>No Male Alleles Present</td>
<td>No Male Alleles Present</td>
</tr>
<tr>
<td>1:1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:2</td>
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<tr>
<td>1:128</td>
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<td>0</td>
</tr>
<tr>
<td>1:256</td>
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<td>0</td>
</tr>
<tr>
<td>1:512</td>
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<td>0</td>
</tr>
<tr>
<td>1:1024</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Erase Differential Separation

Complete, clean, male DNA profiles to the 1:16 dilution

PBSO Differential Separation

Complete, clean, male DNA profiles to the 1:1 dilution

Non-Sperm Cell Fractions (Note: data not shown)

- Full mixtures to the 1:256 dilution
- Full female profiles in all dilutions
- Discrepant male minor contributor appearing at the 1:8 dilution

Conclusions

- Sensitivity-Stochastic/Mixture: The Erase separation protocol had comparable sensitivity to the current PBSO differential separation protocol
- Repetitiveness/Reproducibility/Known-Concordance: The Erase separation protocol did not introduce any contamination into the extraction step
- Precision/Accuracy: The Erase separation protocol had comparable results to the current PBSO differential separation protocol

Acknowledgements

This project benefited from the input of Amy McGuckian, Julie Sikorsky, Cathy Cothran, Dr. Cecelia Crouse, Season Seferyn, and Dr. Pamela Staton, all of whom provided assistance and critiques of the presentation and these studies. Thanks are also given to Sharma Saunders who provided support in the production of this poster. The author also thanks the analysts and employees of the Palm Beach County Sheriff’s Office Forensic Biology Unit, who gave valuable advice, encouragement, and made the experience within their laboratory worthwhile.

References

6. 6.2 (2012): 282
7. Annexed Analytical Methods: The Erase separation protocol had comparable results to the current PBSO differential separation protocol.
8. Note: data not shown