



Internal Validation of the PTC Erase Sperm Isolation Kit



FORENSIC SCIENCE

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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 QIAGEN® EZ1® DNA Investigator® Kit in conjunction with the QIAGEN® EZ1® Advanced XL for extraction. This method 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 to or better than the current method.

Materials & Methods

Samples

Sensitivity/Stochastic/Mixture: serial dilution of liquid semen and water to include 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, and 1:1024 added to a swab with 25 µL female blood

Known-Concordance/Reproducibility/Repeatability: previously typed differential samples extracted in duplicate and run on different EZ1® instruments

Precision/Accuracy: serial dilution of liquid semen and water to include 1:8, 1:16, 1:32, 1:64, and 1:128 added to a female buccal swab obtained by rubbing the inside of both cheeks and extracted in duplicate on an EZ1® instrument

Workflow

Pre-Processing: Differential separation with PTC Erase Sperm Isolation Kit protocol and PBSO differential separation protocol

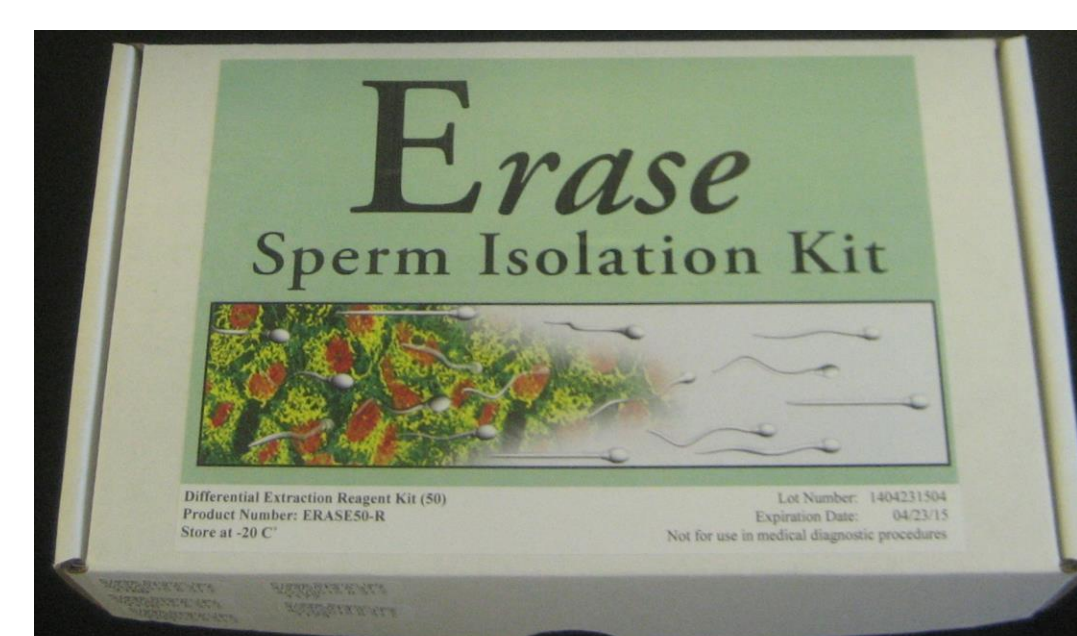
Extraction: Qiagen® EZ1® DNA Investigator Kit on the Qiagen® EZ1® Advanced XL

Quantification: Promega® Plexor® HY and an AB® 7500 Real-Time PCR System

Amplification: Promega® PowerPlex® 16 with an AB® GeneAmp® PCR System 9700

Capillary Electrophoresis: Applied Biosystems® 3130xl Genetic Analyzer

Analysis: Applied Biosystems® GeneMapper® ID-X Software v1.3.



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

Sensitivity (Semen : Water) On Female Blood Swabs	Erase Differential Separation		PBSO Differential Separation	
	# Carry-Over Alleles Present	# Male Alleles Dropped Out	# Carry-Over Alleles Present	# Male Alleles Dropped Out
1:1	0	0	0	0
1:2	0	0	0	0
1:4	0	0	0	0
1:8	0	0	2	0
1:16	0	0	0	0
1:32	3	0	5	0
1:64	2	0	14	0
1:128	10	0	15	0
1:256	21	0	21	0
1:512	21	1	19	10
1:1024	8	7	21	2

Erase Differential Separation

- Complete, clean, male DNA profiles to the 1:16 dilution
- Complete, male DNA profiles to the 1:256 dilution; male drop-out began to occur at the 1:512 dilution
- Female carry-over alleles began to appear at the 1:32 dilution
- Male alleles began to drop below stochastic threshold in the sperm fraction at the 1:256 dilution (note: data not shown)

PBSO Differential Separation

- Complete, clean, male DNA profiles to the 1:4 dilution
- Complete, male DNA profiles to the 1:256 dilution; male drop-out began to occur at the 1:512 dilution
- Female carry-over alleles began to appear at the 1:8 dilution
- Male alleles began to drop below stochastic threshold in the sperm fraction at the 1:256 dilution (note: data not shown)

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

- Full mixtures to the 1:256 dilution
- Full female profile present in all dilutions
- Discernable male minor contributor appearing at the 1:8 dilution

Repeatability/Reproducibility/Known-Concordance Studies

- All profiles obtained were concordant with the expected profiles (note: data not shown)

Contamination Study

- All 12 negative control samples showed no contamination at the extraction step (note: data not shown)

Precision/Accuracy Study

Table 2: Summary of precision/accuracy study results for the sperm fraction separated using both the PTC Erase Sperm Isolation Kit and PBSO's differential separation protocol

Sensitivity Ratio (Semen : Water) On Female Buccal Swabs	Erase Differential Separation		PBSO Differential Separation	
	# Carry-Over Alleles Present	# Male Alleles Dropped Out	# Carry-Over Alleles Present	# Male Alleles Dropped Out
1:8	11	0	19	0
1:16	2	0	17	0
1:32	2	0	19	0
1:64	19	0	19	0
1:128	14	0	19	0

- No clean separations, but complete male profiles obtained at all dilutions
- Greater variation among sensitivity ratios for Erase regarding carry-over

Conclusions

- **Sensitivity-Stochastic/Mixture:** The Erase separation protocol had comparable sensitivity to the current PBSO differential separation protocol
- **Repeatability/Reproducibility/Known-Concordance:** The Erase separation protocol was able to repeatedly reproduce a profile concordant with the known profile
- **Contamination:** 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
- Approximate cost per sample
 - Erase protocol (consumables included in kit): \$ 13.80
 - PBSO protocol (not including consumables): \$ 4.43
- Approximate time per sample
 - Erase protocol: 90 minutes
 - PBSO protocol: 40 minutes
- **The Erase Sperm Isolation Kit produces comparable results to the differential separation method currently utilized by PBSO**
- **If utilized at PBSO, the main advantage of the Erase Sperm Isolation Kit would be the reduction of sample handling and processing steps due to the elimination of sperm cell pellet washes**

References

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