The Validation of a Manual Selective Degradation Method of Differential Extraction

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Abstract

The present research aims to validate the manual application of the selective degradation method of a differential extraction using the Erase Sperm Isolation Kit and similarly made in-house DNase reagents. For this research, a comparison of the Erase reagents and DNase reagents was made using serial dilution swabs as well as mock casework swabs already processed with KSPCL's current method of differential extraction, using the QIAcube. A sensitivity study was performed to assess how well both reagents recovered sperm cell DNA at different seminal dilutions. The sensitivity study was reproduced to ensure consistent results were obtained with both reagents when performed by the same analyst. A mock casework study was performed to assess repeatability with both reagents when compared to the QIAcube. The results of this research illuminate some of the advantages that accompany selective degradation but also highlight variations within the data that supports justification for further research before implementation as methods of manual differential extraction at KSPCL.

Introduction

- Sexual assault evidence makes up a large portion of the caseload encountered at many forensic DNA laboratories[1]
- Sexual assault evidence is commonly received in the form of swabs, most often composed of a mixture of female epithelial cells and male sperm cells
- For genetic STR analysis and interpretation of these samples, it is beneficial that the female epithelial cells be separated from the male sperm cells allowing for independent analyses of both fractions[1]
- The gold standard for separation is a method known as differential extraction
- Differential extraction involves multiple pipetting steps, making this method time consuming and more prone to introducing error and contamination
- Due to the nature of the evidence, differential extraction often fails to eliminate all female epithelial cell DNA leaving analysts with a mixture to interpret
- With many forensic DNA laboratories experiencing an increasing demand for the analysis of sexual assault evidence[2], the labor intensive and time-consuming nature of differential extraction, and a shortage of DNA analysts in many forensic DNA laboratories labs are being faced with an increase in backlog[2]
- Selective degradation is a method of differential extraction that requires the addition of a nuclease which eliminates extra pipetting steps
- Selective degradation becomes more time efficient, minimizes the chances for error and contamination, and results in male STR profiles that are easily interpretable
- This research aimed to validate the use of the selective degradation method for a manual differential extraction using both Erase and DNase reagents

Materials & Methods

- For all studies performed, the manual version of the Erase Sperm Isolation Kit or similarly made in-house DNase reagents were used and compared to the results obtained from the QIAcube
- For the sensitivity study and sensitivity study reproduced, the serial dilution swabs used were made by pipetting 1:3-1:2187 serial dilutions of semen and water onto female buccal swabs
- For the mock casework study, mock casework swabs previously collected from 3 individuals at varying post-coital time intervals and previously processed using the QIAcube were used

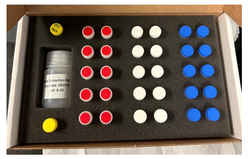

Fig. 1- Erase Sperm Isolation Kit with 30mL of extraction buffer, 2 tubes of 300µL of ProK, 10 tubes of 100µL of Solution 1 (nuclease activator), 10 tubes of 100µL of Solution 2 (the nuclease), and 10 tubes of 100µL of Solution 3.

Fig. 2- Erase Sperm Isolation Kit with dolphin nose tubes (Tube A), pink tubes for female fractions (Tube B), blue tubes for male fractions (Tube C), and spin baskets.

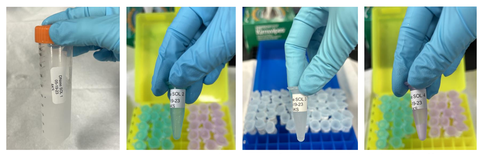

Fig. 3-6- In House DNase Reagents with 100mL of Solution 1 (lysis/digest buffer) containing Triton X-100, EDTA, and ProK, 5mL of Solution 2 (nuclease activator) containing $CaCl_2$ and $MgCl_2$, 8.3 mL of Solution 3 (the nuclease) containing EDTA and DNase I, and 5mL of Solution 4 containing DTT and ETDA.

Results

Quantitation Data

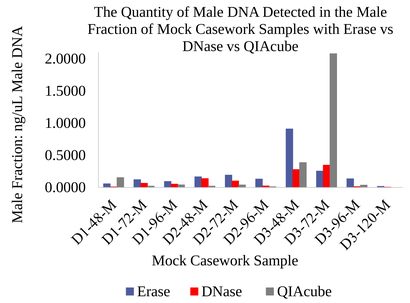

Fig. 7– Bar Graph of the Quantity of Male DNA Detected in the Male Fraction of Mock Casework Samples Based on Differential Extraction Method.

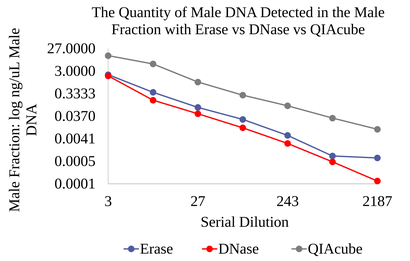

Fig. 8– Scatter Plot of the Quantity of Male DNA Detected in the Male Fraction with Erase vs DNase vs QIAcube for Sensitivity Study 1 Using a Logarithmic Scale of Base 3. *Both axes were subjected to a log base 3 scale to put all the data on the same scale. The X-axis values correspond to the serial dilution letters (A-G) used for labeling in the studies conducted above (Ex. Serial dilution 3= "A").*

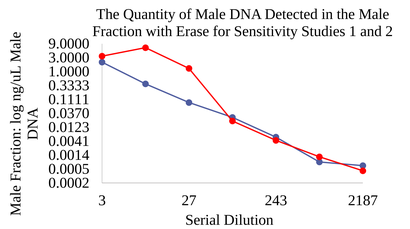

Fig. 9– Scatter Plot of the Quantity of Male DNA Detected in the Male Fraction with Erase for Sensitivity Studies 1 and 2 Using a Logarithmic Scale of Base 3. *Both axes were subjected to a log base 3 scale to put all the data on the same scale. The X-axis values correspond to the serial dilution letters (A-G) used for labeling in the studies conducted above (Ex. Serial dilution 3= "A").*

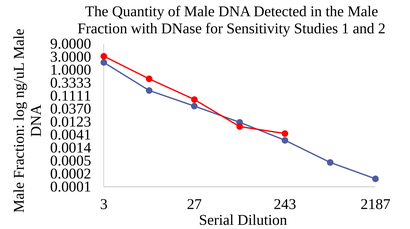

Fig. 10– Scatter Plot of the Quantity of Male DNA Detected in the Male Fraction with DNase for Sensitivity Studies 1 and 2 Using a Logarithmic Scale of Base 3. *Both axes were subjected to a log base 3 scale to put all the data on the same scale. The X-axis values correspond to the serial dilution letters (A-G) used for labeling in the studies conducted above (Ex. Serial dilution 3= "A").*

Capillary Electrophoresis Data

Mock Casework Sample	Av. PHR QIAcube	Av. PHR Erase	Av. PHR DNase	% alleles QIAcube	% alleles Erase	% alleles DNase
D1-48-F	0.89	0.86	0.86	100%	100%	100%
D1-72-F	0.87	0.88	0.86	100%	100%	100%
D1-96-F	0.88	0.89	0.84	100%	100%	100%
D2-48-F	0.93	0.90	0.90	100%	100%	100%
D2-72-F	0.95	0.94	0.92	100%	95%	100%
D2-96-F	0.90	0.92	NA	100%	100%	NA
D3-48-F	0.91	0.89	0.84	100%	100%	78%
D3-72-F	0.90	0.89	0.89	100%	100%	100%
D3-96-F	NA	0.89	0.90	NA	100%	100%
D3-120-F	X	0.89	0.90	X	100%	100%
D1-48-M	0.89	0.88	0.86	100%	100%	97%
D1-72-M	0.56	0.90	0.88	100%	100%	100%
D1-96-M	0.72	0.87	0.91	100%	100%	100%
D2-48-M	0.51	0.86	0.84	100%	100%	100%
D2-72-M	0.49	0.65	0.81	98%	88%	100%
D2-96-M	0.58	F	0.85	84%	F	100%
D3-48-M	0.87	0.87	0.84	100%	100%	100%
D3-72-M	0.87	0.80	0.86	100%	100%	100%
D3-96-M	F	0.80	0.82	F	100%	100%
D3-120-M	X	0.69	0.72	X	91%	86%

Table 1– Average Peak Height Ratios and % Alleles Recovered from Mock Casework Samples When Using QIAcube vs Erase vs DNase. *Data were generated by using reference profiles in comparison to electropherograms produced during this study as well as by using KSP's genotypes table excel spreadsheet. Pink boxes represent all mock casework female fractions while blue boxes represent all mock casework male fractions. "NA" was recorded for any instance that no data was recovered after quantitation. "X" was recorded for any instance that the specific sample was not run with the specific method of differential extraction. "F" was recorded for any instance that the DNA profile in the male fraction matched the female contributor.*

Serial Dilution	Average Av. PHR Erase	Average Av. PHR DNase	Average % alleles Erase	Average % alleles DNase
A-F	0.90	0.90	100%	100%
B-F	0.89	0.90	100%	100%
C-F	0.90	0.89	100%	100%
D-F	0.90	0.89	100%	100%
E-F	0.88	0.88	100%	100%
F-F	0.89	0.90	100%	100%
G-F	0.91	0.90	100%	100%
A-M	0.87	0.84	100%	100%
B-M	0.86	0.85	100%	99%
C-M	0.90	0.90	100%	100%
D-M	0.84	0.86	100%	88%
E-M	0.81	0.85	100%	89%
F-M	0.88	NA	71%	NA
G-M	0.97	NA	23%	NA

Table 2– Average Peak Height Ratios and Average % Alleles Recovered from Serial Dilutions for Sensitivity Study 1 when Using Erase vs DNase. *Data were generated by using reference profiles in comparison to electropherograms produced during this study as well as by using KSP's genotypes table excel spreadsheet. Pink boxes represent all serial dilution female fractions while blue boxes represent all serial dilution male fractions. "NA" was recorded for any instance that no data was recovered after quantitation.*

Discussion

Mock Casework Study

- In comparison to the QIAcube, Erase recovered more male fraction male DNA in 7 samples while DNase recovered more in 5 samples (Fig. 7)
- DNase recovered lower than expected female total human DNA than the QIAcube which could be due to lack of inversion of the master mix
- Average PHRs were consistent among all female and male fraction samples except for lower PHRs from the QIAcube with some male fraction samples (Table 1)
- Erase and DNase recovered 100% of alleles with more samples than the QIAcube (Table 1)

Sensitivity Study

- The QIAcube recovered more male fraction male DNA than both Erase and DNase with all 7 serial dilutions (Fig. 8) which could be due to the serial dilution swabs not being representative of true casework swabs
- Average PHRs for both Erase and DNase are consistent (Table 2)
- Erase recovered 100% of alleles with more serial dilutions than DNase (Table 2)

Sensitivity Study Reproduced

- For Erase, more male fraction male DNA was recovered in the reproduced sensitivity study than in the first sensitivity study with 4 serial dilutions (Fig. 9)
- For DNase, more male fraction male DNA was recovered in the reproduced sensitivity study than in the first sensitivity study with 4 serial dilutions (Fig. 10)

Overall

- Erase consistently recovered more male fraction male DNA in all studies in comparison to DNase which could be due to a greater $CaCl_2$ concentration in Solution 2 of the DNase reagents
- The quality of most profiles were sufficient for all studies performed
- Erase and DNase were both more efficient at eliminating female DNA from the male fraction in both the first sensitivity study and the mock casework study

Statistics

- A t-test was performed using Excel's paired two sample t-test for means function on the data from sensitivity study 1

Mean DNA Recovery for Serial Dilutions A-G	Erase vs DNase	Erase vs QIAcube	DNase vs QIAcube
Male Fraction Male DNA	Failed to reject H_0	H_0 rejected 3 times	H_0 rejected 3 times
Male Fraction Total Human DNA	H_0 rejected 2 times	H_0 rejected 1 time	H_0 rejected 1 time
Female Fraction Male DNA	H_0 rejected 1 time	H_0 rejected 1 time	Failed to reject H_0
Female Fraction Total Human DNA	H_0 rejected 1 time	H_0 rejected 2 times	H_0 rejected 2 times

Table 3- Rejection of H_0 after comparisons between the mean DNA recoveries for male fraction male DNA, male fraction total human DNA, female fraction male DNA, and female fraction total human DNA for the 3 methods where H_0 was no difference between the DNA recovery means within the datasets of two different methods and H_A was any difference between said means.

Conclusion

This study highlights the promising nature of using Erase and DNase reagents for the selective degradation method of a manual differential extraction when compared to using the QIAcube, but also suggests the need for further research and improvements of the methods. A lot of variability was seen in the data of this research and because validations take a lot of time and resources, KSPCL was unable to continue this validation at this time. Once more research can be conducted, the implementation of selective degradation would provide KSPCL with a method of differential extraction that is not only cost and time efficient but has been proven by this research to give typable STR profiles that are as readily interpretable as the profiles produced using the QIAcube.

References

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Acknowledgements

I thank Kentucky State Police Central Laboratory for allowing me to join them this summer and conduct this research using their laboratory and resources. I also thank Dr. Waugh and the Marshall University Forensic Science Graduate Program for giving me this opportunity.