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Development of a Simple Differential Extraction Method for Analysis of Forensic Sexual Assault Evidence Using the RapidHIT ID™

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INTRODUCTION

Rapid DNA represents a novel forensic technology, wherein the sample analysis process is fully automated, necessitating no human intervention. This technology allows for short-tandem repeat (STR) DNA profiles to be generated in as little as 90 minutes, as opposed to the hours to days of analysis and interpretation required in casework laboratories. Thus far, the FBI has only enabled the use of this technology for the analysis of reference samples (blood or saliva) with STR profiles obtained being entered into CODIS. Other law enforcement agencies use Rapid DNA analysis outside of CODIS to significantly impact investigations related to incidences of human trafficking and the recovery of unidentified human remains. Currently, efforts are being made to allow the use of Rapid DNA analysis for crime scene samples, with an expected timeline for implementation of 2025. A large amount of the evidence submitted to forensic biology laboratories is in the form of sexual assault kits (SAKs). However, evidence collected from these kits is rarely the target of Rapid DNA analysis due to the need for differential extractions (DEs), a technique that rapid DNA instruments cannot perform.

The current project sought to optimize an off-instrument DE method to analyze SA evidence with the RapidHIT ID™ system, using the new RapidINTEL™ Plus cartridges. Thus far, we have developed a simple, accelerated standard DE workflow utilizing a 1-hour differential lysis for the preferential lysis of epithelial cells. Here we demonstrate the ability to obtain single-source male DNA profiles from as little as 1 µL semen admixtures with vaginal epithelial cell samples as well as post-coital (PC) samples, with the full analytical sensitivity of the DE method still being evaluated. For laboratories that will be using standard-sized cotton swabs for their analysis, parallel experiments using lysis volumes of both 'general' and 'specialized' protocols are being conducted to optimize sample analysis.

SAMPLES

Samples (vaginal secretions and semen) were collected using procedures approved by the University of Central Florida's Institutional Review Board. Informed consent was obtained from all donors. Accuracy of obtained profiles verified by comparison to reference profile.

CONCLUSIONS

- Single-source male profiles were obtained from mock vaginal-semen mixtures with as little as 1 µL of semen with no male drop-out.
- Full male profiles were obtained using only 5 µL of prepared semen fraction (out of 30 µL (i.e. 1/6th portion) added onto a HydraFlock® micro (HFM) swab.
- 25 µL of the sperm fraction is left remaining for re-analysis (rapid or standard workflows).
- The success of the developed method was demonstrated with *bona fide* post-coital cervicovaginal and drainage samples collected 18-36 hours after intercourse, with full single source male profiles obtained.
- Further work will be focused on protocol optimization to further reduce potential epithelial cell DNA carry-over as well as testing additional post-coital samples (more donors, later time intervals).

ACKNOWLEDGEMENTS

The authors would like to acknowledge the anonymous donors for their body fluid and PC sample contribution. Other acknowledgments include UCF's Department of Chemistry and the National Center for Forensic Science. A Sponsored Research Agreement with ThermoFisher Scientific™ supported this research work.

WORKFLOW

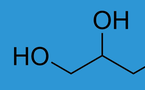
Whole cotton swab placed in PrepFiler *Express*™ tube

400 µL stain extraction buffer (SEB) & 10 µL proK added

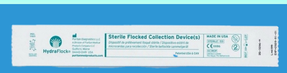
56°C 1 hr
500 rpm

13,000 rpm 5 min
Transfer NSP
Store substrate

Wash x 2
200 µL SEB
Discard SEB



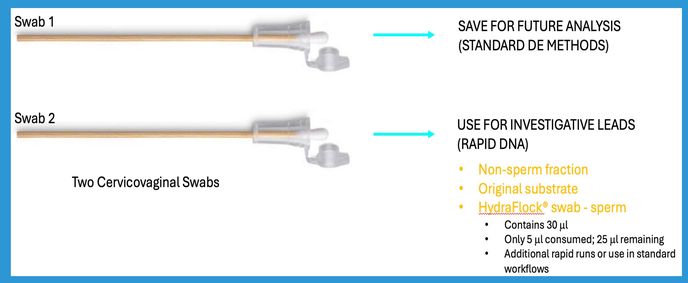
Add 30 µL LTE buffer & 0.75 µL 1-Thioglycerol for SP lysis; incubate at 56°C for 5 min



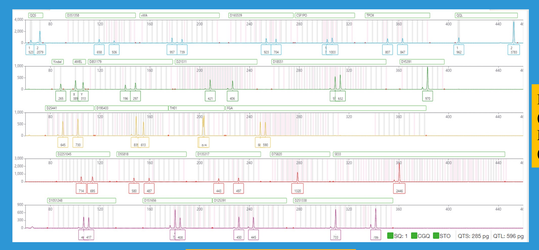
Pipette 5 µL SP aliquot onto Puritan® HydraFlock® micro (HFM) sterile swab

RapidHIT™
RapidINTEL™ Plus
RapidLINK™ v2.0
specialized protocol

USE OF RESIDUAL SAMPLE

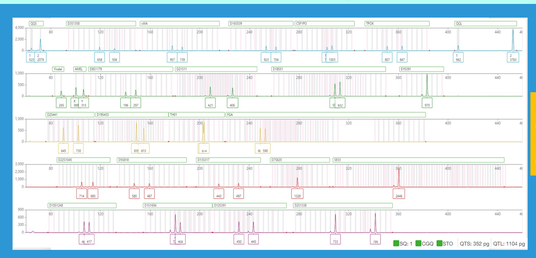


VAGINAL-SEMEN (1 µL) MOCK MIXTURES



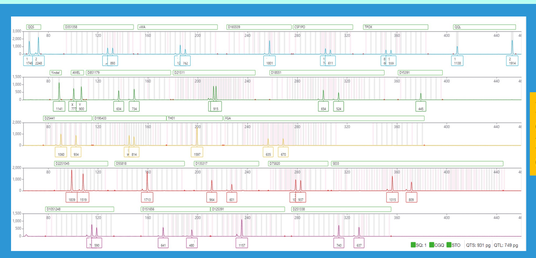
Female Alleles (X): 0
Male Dropout (*): 0

Donor Set #1



Female Alleles (X): 0
Male Dropout (*): 0

Donor Set #2



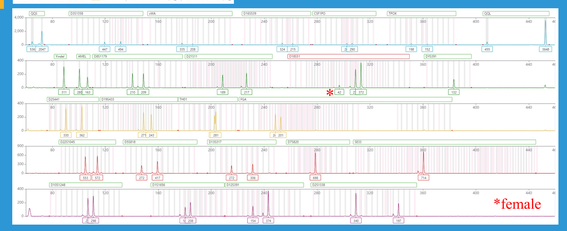
Female Alleles (X): 0
Male Dropout (*): 0

Donor Set #3

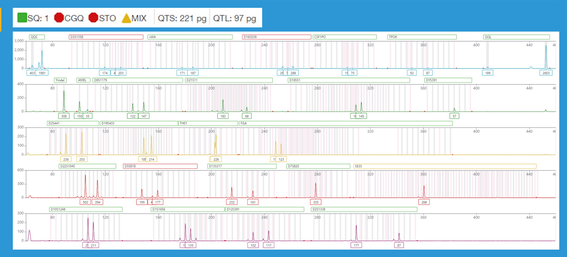
REPRODUCIBILITY

Three 5 µL aliquots from same sperm fraction sample (VS-SE 1µL mixture)

#1



#2



#3

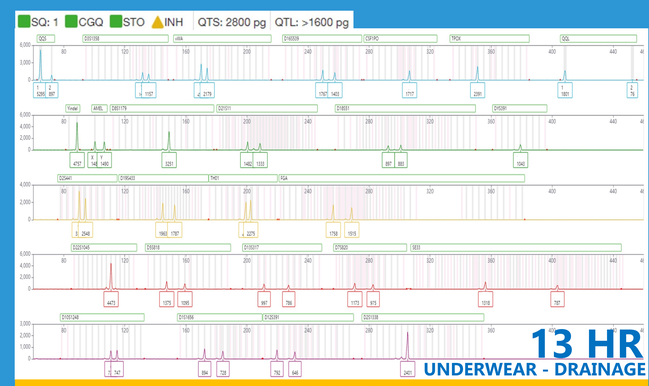
12 – 36 HR *BONA FIDE* POST COITAL SAMPLES

Female Alleles (*): 4 Male Dropout (*): 0

PC Sample	S. Auto (ng/µL)	Total Human (ng)	Y (ng/µL)	Total Male (ng)	Total Female (ng)	F/M Ratio
18 hr	160	4800	9.7	291	4509	~16:1
36 hr	301	9030	7.9	237	8793	~37:1

The second swab for each time interval was extracted using a non-differential PrepFiler *Express*™ extraction (30 µL elution) and quantitated with the Quantifiler™ Trio kit in order to obtain an approximate F/M ratio for the post coital samples. Even with a ~37:1 F/M ratio, a single source male profile was obtained.

Female Alleles (*): 0 Male Dropout (*): 0



Female Alleles (*): 0 Male Dropout (*): 0