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# Development of a Simple Differential Extraction Method for Analysis of Forensic Sexual Assault Evidence Using the RapidHIT ID<sup>TM</sup>

#### 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 offinstrument DE method to analyze SA evidence with the RapidHIT ID<sup>TM</sup> system, using the new RapidINTEL<sup>TM</sup> 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 singlesource 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  $\mu$ L of semen with no male drop-out.
- Full male profiles were obtained using only 5  $\mu$ L of prepared semen fraction (out of 30 μL (i.e. 1/6<sup>th</sup> portion) added onto a HydraFlock<sup>®</sup> 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

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> Whole cotton swab placed in PrepFiler  $Express^{TM}$ tube

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



## WORKFLOW



Wash x 2200 µL SEB **Discard SEB** 



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



# REPRODUCIBILITY

L aliquots from same sperm fraction sample (VS-SE 1µL mixture)											
CGQ 🔺 STO	QTS: 291 pg	QTL: 248 pg									
D351358	wwa Tść	200	240	CSF1PO       280     320	(TPOX 360	400	440 44				
447     494       Yindel     AMEL     D851179       120     120		<u>208</u> <u>21511</u> 200	240	280 320	198 152 DV\$391 360	455 400	3648 440 44				
1 D195433	тн тн тбо	200	240	280 320		400					
330 362 D2251045 120	271 243 [D55818 160	281 D135317 200	2 201 D75820 240		360	400	440 44				
1248 120 120 120	272 417 DIS1656	272 D125391	240	698 D251338 280 320 340	360	400 *fer	nale				
Image: CGQ STO AMIX QTS: 221 pg QTL: 97 pg											
D3\$1358 120	(wwa160	200	<u>65539</u> 240	CSFIPO       280     320	(TPOX) 360	<u>(QQL</u> 400	440 46				
174 d 203		187 [D21511 200	24 7 288 D18551	280 320	92 87 DYS391 360	400	2603 440 4(				
308 150 35 D195433 120 236 253	122 147 TH	200 FGA	240	280 320	360	400	440 44				
502 394	160 195 4 177	D135317 200	240 240	280 320 335	360	400	440 46				
	D151656	200 135	240	280 320	360	400	440 44				
CGQ STO		238 pg QTL: 21	4 pg								
D351358	(wa 160	200 [D	240		360	400	440 44				
indel AMEL D851179	j 255 	5 313 [D21511 200	22 \$ 238 D18551	280 320	202 202 DYS391 360	397 400	1977 440 4é				
235 359 162 1 D195433	197 132 TH	289 [1] 01 [FGA	2/0	× 60 3 198	[170]	400	4/0				
305 273 D2251045	274 295 D55818	200 [ 176 [ 175317	1 164 D75820	200 320	300	4UU	440 4				
120 	160	200 328 D125391	240	280 320 614 D251338	360 	400	440 46				
120	160	200	240	280 320	360	400 *fe	<sup>440</sup> 4				





# VAGINAL-SEMEN (1 µL) MOCK MIXTURES



### **12 – 36 HR BONA FIDE POST COITAL SAMPLES**





# **USE OF RESIDUAL SAMPLE**

SAVE FOR FUTURE ANALYSIS (STANDARD DE METHODS)

#### USE FOR INVESTIGATIVE LEADS RAPID DNA)

- HvdraFlock<sup>®</sup> swab sperm
- Contains 30 ul
- Only 5 µl consumed: 25 µl remainin

. Auto ng/μL)	Total Human (ng)	Υ (ng/μL)	Total Male (ng)	Total Female (ng)	F/M Ratio
160	4800	9.7	291	4509	~16:1
301	9030	7.9	237	8793	~37:1

The second swab for each time interval was extracted using a nondifferential PrepFiler *Express<sup>TM</sup>* extraction (30 µL elution) and quantitated with the Quantifiler<sup>™</sup> 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.

