Simplified DE Method for the RapidHIT™ ID to Obtain Investigative Leads from Sexual Assault Evidence

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INTRODUCTION

Rapid DNA fully integrates sample analysis workflow from extraction to capillary electrophoresis, generating autosomal STR profiles in as little as 90 minutes without the need for a DNA laboratory. The FBI allows DNA profiles developed from *reference samples* on rapid DNA instruments to be uploaded to CODIS, but not from *crime scene samples*. Reference samples collected from arrestees at some booking stations are being uploaded to CODIS and quickly searched against the DNA Index of Special Concern (DISC) established by the FBI for comparison to crime scene profiles from unsolved homicide, sexual assault, kidnapping and terrorism cases. This practice is expanding nationwide because of its ability to link arrestees to unrelated crimes while he/she is still in custody.

Although the FBI does not currently allow DNA profiles generated from *crime scene samples* on rapid DNA instruments to be uploaded to CODIS, they are working with vendors to enable this capability in early 2025. In the meantime, many law enforcement agencies have been using rapid DNA outside of CODIS to substantially impact criminal investigations, human trafficking, and the identification of human remains. While most of this work has been done with blood and saliva cases, rapid DNA is rarely used for sexual assault cases because rapid DNA instruments do not perform differential extractions. We sought to develop an off-instrument differential extraction method that was compatible with non-technical users and rapid DNA instruments to enable law enforcement to take advantage of the speed of rapid DNA in sexual assault cases.

The goal of the current work was to develop simplified DE methods for the RapidHIT™ ID system for use with semen-containing evidence found in sexual assault investigations. The methods developed are designed to be used for investigative leads in a laboratory or potentially in point-of-collection use environments. The outcome is a simple workflow that utilizes a 1-hour differential lysis to preferentially lyse epithelial cells leaving sperm cells intact. After a few brief washes, the epithelial cell fraction is separated, and the remaining sperm pellet can then be collected with a sterile swab and run on the RapidHIT™ ID system. Using this simplified DE method, single source male DNA profiles can be obtained from as little as 1 µl of semen, with the full sensitivity of the method still being evaluated. Mock mixtures using both buccal and vaginal epithelial cells were evaluated representing possible casework scenarios of vaginal and oral assaults. Successful results were also obtained from volunteer donated 12- and 24-hr post-coital samples.

SAMPLES

All samples were collected from volunteers using procedures approved by the University of Central Florida's Institutional Review Board. Informed consent was obtained from each donor.

FUTURE WORK

- Continue to optimize protocol for improved profile recovery with small amounts of semen
- Test more *bona fide* post coital samples, including samples 3 – 5 days after intercourse.

ACKNOWLEDGEMENTS

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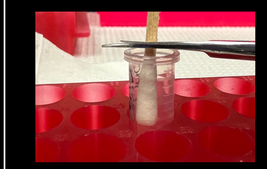
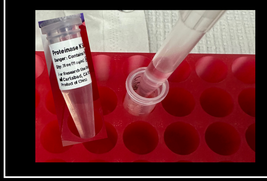
WORKFLOW

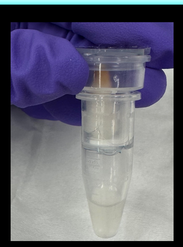


Whole cotton swab placed in PrepFiler *Express*™ tube

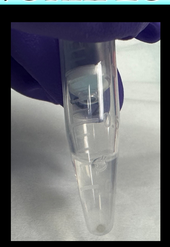


400 ml stain extraction buffer (SEB) & 10 ml proK added

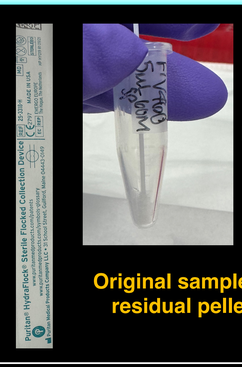
56°C 1 hr
500 rpm



13,000 rpm 5 min
Transfer NSP
Store substrate



Wash x 2
250 ml SEB
Discard SEB



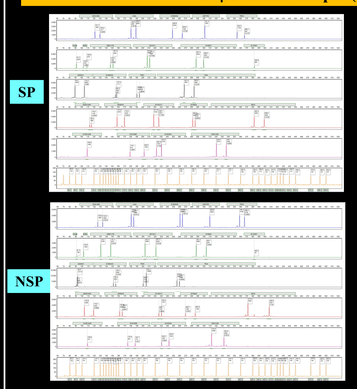
Sperm pellet collected with a Puritan® HydraFlock® sterile swab

Original sample tube saved since residual pellet may be present

RapidINTEL™ cartridge

SENSITIVITY

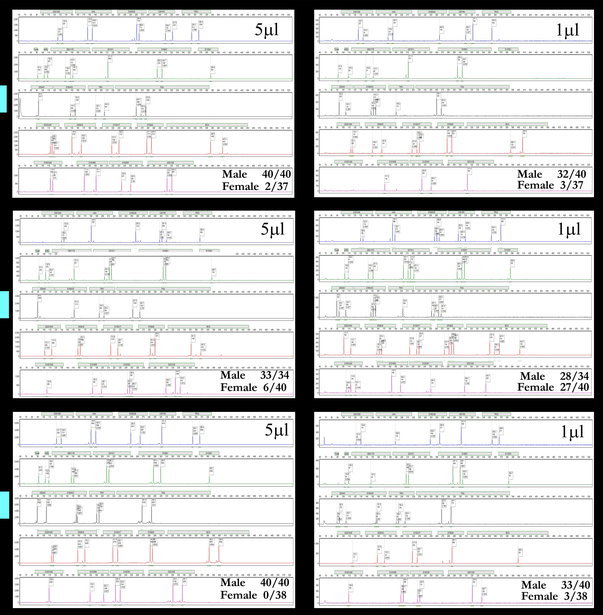
15 µl semen sample (buccal ep mix)



Locus	Sperm	Non-Sperm
D3S1358		
vWA		
D16S539		
CSF1PO		
TPOX		
Yindel		
AMEL		
D8S1179		
D21S11		
D18S51		
DYS391		
D2S441		
D19S433		
TH01		
FGA		
D22S1045		
D5S818		
D13S317		
D7S820		
SE33		
D10S1248		
D1S1656		
D12391		
D2S1338		

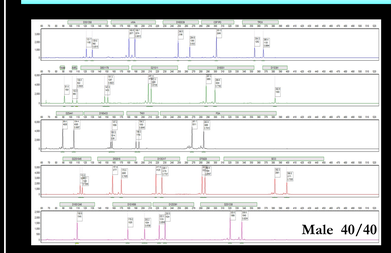
Gold square = profile matched reference (no drop out)

5, 1 µl semen/ep mixtures – 3 donor sets



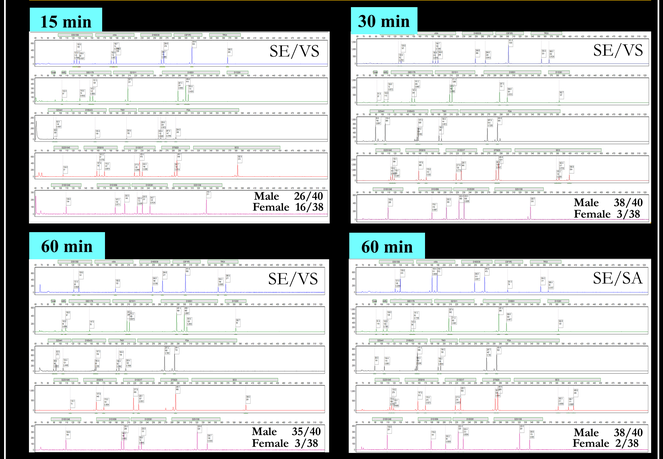
Successful results have been obtained from 15, 10, 5 and 1 µl of semen. With 1µl, as expected, male allele drop out and the presence of female alleles is observed with more frequency. However, significant partial to full profiles have been obtained for all samples.

LYSIS TIME



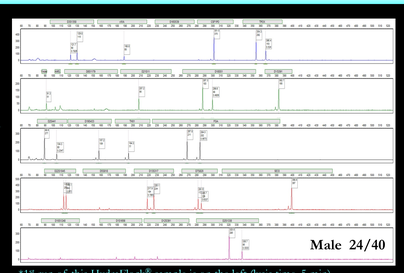
5 min lysis
With higher semen volumes, single source male profiles can be obtained with a 5 min lysis

1 µl semen/ep mixtures



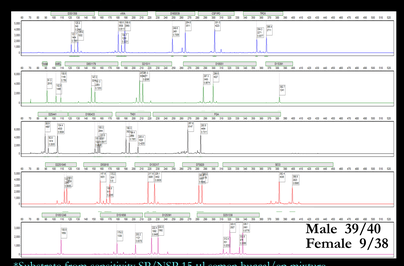
While successful results can be obtained with < 1 hr lysis time, due to improved profile recovery for 1 µl samples with the 1 hr lysis time and the critical nature of the sexual assault samples, 1 hr was selected as optimal.

USE OF RESIDUAL SAMPLE



*1st run of this HydraFlock® sample is on the left (lysis time, 5 min)

HydraFlock® swabs can be removed from RapidINTEL™ cartridge after 1st run, and re-run in a new cartridge. Some genetic material may be left on the swab. Alternatively, that swab can be extracted using standard lab DE protocols.



*Substrate from sensitivity SP/NSP 15 µl semen buccal/ep mixture

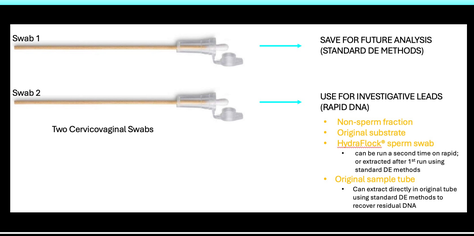
The original cotton swab substrate remaining after sperm pelleting can be run directly in a RapidINTEL™ cartridge. Sperm and non-sperm cells remain on the substrate as is seen with standard DE methods as well.

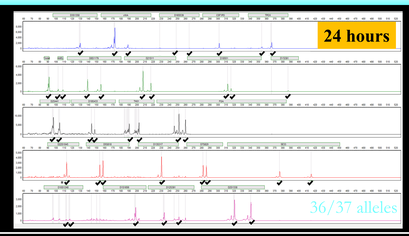
Ep Cell Type	Vol of Semen	Sample	S.Auto (ng/ul)	Y (ng/ul)	STR profile
Vaginal	5ul	SP	7.9	6.4	Full
		2nd Extraction	0.48	0.37	Full
	1ul	SP	0.83	0.66	Full
		2nd Extraction	0.05	0.02	Low level mixture
Buccal	5ul	SP	3.9	1.9	Full
		2nd Extraction	0.24	0.21	Full
	1ul	SP	0.93	0.62	Full
		2nd Extraction	0.16	0.15	Full

SP = HydraFlock sperm pellet swab
2nd Extraction = Extraction of original tube after SP collected

The original sample lysate tube can be swabbed a second time to recover any residual sperm pellet that may remain in the tube or a standard PrepFiler™ *Express* extraction can be performed directly in the original tube. ~10% of the sperm pellet DNA can sometimes be remaining in the tube.

24-HR POST COITAL SAMPLE





- Male profile obtained from a cervicovaginal sample collected 24 hours after intercourse.
- Male genotype confirmed (check mark indicates male alleles) by comparison to reference profile. Only one allele drop out (*) was observed. No alleles from the female donor were observed.