Securing the Future: Long-term Stability of Y-Screened Sexual Assault Evidence

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INTRODUCTION

Sexual assault and rape continue to be serious issues in the U.S., placing significant demands on forensic DNA laboratories to process evidence efficiently. Traditional analysis methods for sexual assault evidence face challenges, such as limited information from serological screening and lengthy differential extractions. These challenges contribute to longer turnaround times and the backlog of sexual assault kits.

To address these concerns and optimize workflows, Y-screening can be performed with quantification kits targeting human male-specific markers. As recommended by SWGDAM and NIJ, this Y-screening approach can reduce upfront sample consumption by streamlining screening and DNA quantification[1,2].

MATERIALS & METHODS

Samples

- Semen spiked onto cotton swabs and cotton fabric (neat, 1:10, 1:100)
- Genuine post-coital vaginal swabs collected 6, 12, 24, or 48 hours post-coital

Substrate Screening Stability (6 months)

- -20°C
- 4°C
- 20°C

Extraction Comparison

- Conventional differential extraction (QIAcube & EZ1)
- Pellet differential extraction following pellet screening

Workflow

- QIAGEN Investigator® Casework GO! kit
- Investigator® Quantiplex® Pro Kit
- Investigator® 24plex QS & Investigator® Argus Y-28 QS kits
- GeneMapper™ ID-X v1.6
- STRmix™ v2.9.1

ACKNOWLEDGEMENTS

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REFERENCES

1. National Institute of Justice. National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach. 2017.
2. Scientific Working Group on DNA Analysis Methods. Report on Y-Screening of Sexual Assault Evidence Kits (SAEKs). 2020.

RESULTS & DISCUSSION

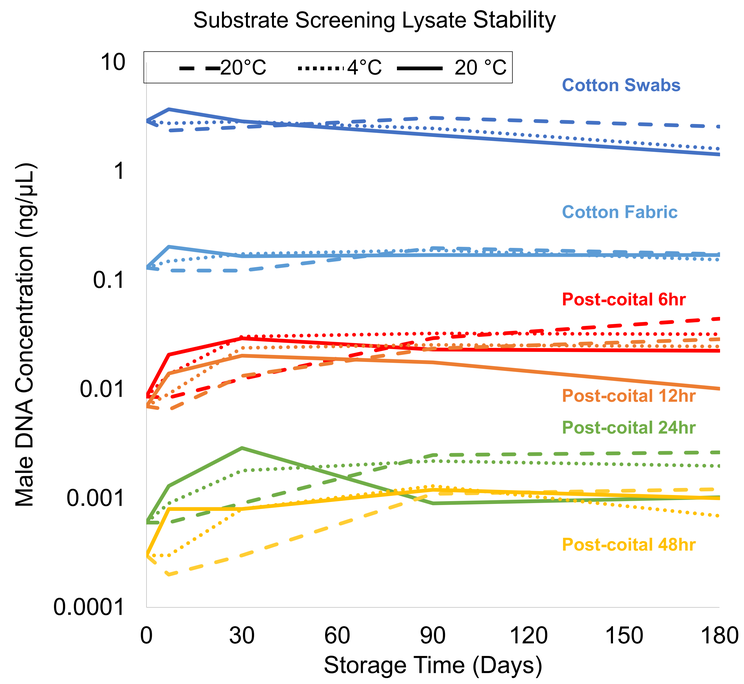

Figure 1. Male DNA stable in substrate screened lysates after 6 months of storage. Across all three storage temperatures (-20°C, 4 °C, and -20 °C), little to no reduction of male DNA was seen. Clusters were mostly due to initial concentration of male DNA.

Substrate Screening Prediction				
Semen	Substrate	Y-Screened Male DNA (ng/µL)	Unique Male a-STR Allele Recovery (%)	Y-STR Allele Recovery
Neat	Swab	2.9051	100	100
1:10		0.1391	100	100
1:100		0.0164	78	100
Neat	Fabric	0.1307	100	100
1:10		0.0161	91	100
1:100		0.0015	11	79

Table 2. Substrate screening predicted success of unique male autosomal allele recovery. Values based on triplicate data. Screened samples with less than 0.0333 ng/µL necessary for PCR target input did not yield in 100% male autosomal allele recovery (highlighted). However, greater success in Y-STR profile recovery was seen in all samples.

Conventional vs. Pellet Differential Extraction					
Sample Number	Post-Coital Interval (hr)	Male DNA (ng/µL)		Unique Male a-STR Allele Recovery (%)	
		Conventional	Pellet	Conventional	Pellet
1	6	2.1842	4.5618	100	100
2	6	0.1795	0.5757	100	100
3	12	0.1657	0.3578	100	100
4	12	0.0137	0.055	85	100
5	24	1.4865	5.857	100	100
6	24	0.5312	1.8349	100	100
7	48	0.0187	0.0818	44	100
8	48	0.1215	0.4686	100	100

Table 1. Modified pellet differential extraction recovers more unique male autosomal alleles than conventional differential extraction. Comparison of conventional and pellet differential extraction shows the difference in removing epithelial fraction prior to differential washing yields greater recovery of unique male autosomal alleles.

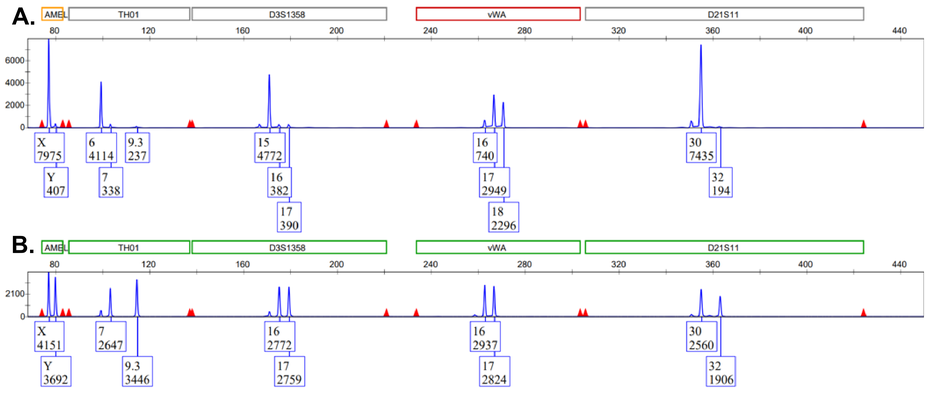

Figure 2. Profile quality difference A. traditional differential extraction and B. pellet differential extraction. In multiple cases, the conventional differential extraction yielded profiles with greater epithelial fraction carryover compared to the pellet differential extraction with better balanced profile.

CONCLUSIONS

- Y-screening with substrate screening is predictive of STR profiling success (**Table 2**).
- Y-screened lysates continue to remain stable after long-term storage (**Figure 1**).
- Differential extraction modified for pellet screening workflow recovers more male alleles and yields cleaner profiles (**Table 1 & Figure 2**).