Assessment of an Automated Differential Separation Utilizing a Novel Nanofiber Filter for Sexual Assault Cases

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Overview

- Background
- Validation Setup
- Validation Workflow
- Results & Conclusions
- Considerations

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Background

Cases involving sexual assault may result in SAK collection
- processed with differential separation
  - manual methods are time consuming and analyst dependent
  - automated methods require funding, but allow throughput increase

In Utah:
- 2017 H.B. 200 – test all kits
- 2018 H.B. 119 – 30 days to submit
- backlog of ~3,000 unsubmitted and/or untested kits

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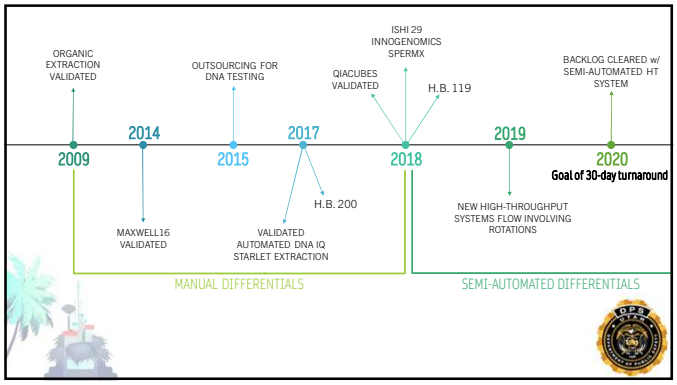

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New Goal

Implementation of robotics resulted in sample throughput increase
- 16 semi-automated samples (M16)
  - 8 sperm fractions, 8 non-sperm fractions
- 72 semi-automated samples (QC)
  - 36 sperm fractions, 36 non-sperm fractions

30-day turnaround poses need for
- more sample processing while maintaining overall quality
- more uniform run conditions for all samples (single run vs. multiple instruments)
- less analyst time in the lab

Fully automated method increases sample throughput
- 192 fully automated samples (AutoLys-SpermX)
  - 96 sperm fractions, 96 non-sperm fractions

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AutoLys Components

AutoLys tubes consist of 2 components
- inner column with outer basket
  - evidence substrates remain within the inner column during digests
  - inner column can be lifted and locked in outer basket ("Lift-and-Lock")
  - liquids flow from inner column into outer basket during centrifugation
- 2d barcode on bottom of tube
  - allows sample tube tracking
  - method generates a final worklist for case records

Nanofiber membrane "SpermX" designed for differential separation
- manual use
- automatable on AutoLys instrument
- captures sperm cells while digested epithelial cells flow through

Images from Hamilton Company

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Validation Setup

Pre-validation Optimization
- **10mg/mL ProK** vs. 20mg/uL ProK + different ratios (epithelial digestion)
- 1 epithelial digestion vs. **2 epithelial digestions**
- G2 buffer vs. TE-4 vs. **sterile water** (sperm washes)

Sample Preparation
- post-coital samples, proficiency tests, casework-type samples, SF serial dilutions, male mixtures, SF on various substrates

Contamination Control
- checkerboard pattern

2 runs on AutoLys
- run 1 = 95 samples
- run 2 = 47 samples

1 run on QIAcubes for comparison
- 36 samples

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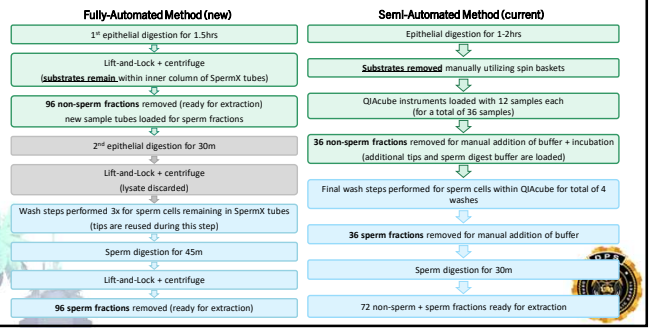
Validation Workflow



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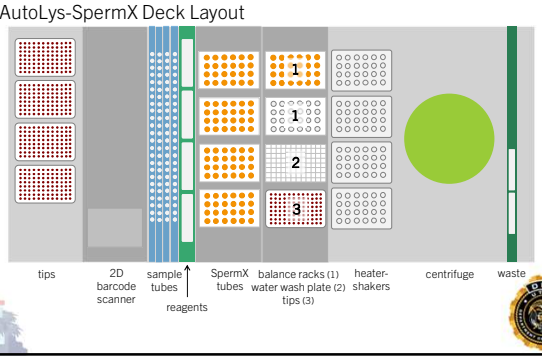
UBFS AutoLys-SpermX Deck Layout



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UBFS AutoLys-SpermX Deck Layout

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Validation Workflow

Procedures following AutoLys-SpermX and QIAcube runs
- Extraction: DNA IQ™ Chemistry
- Quantification: Quantifiler™ Trio Quantification Kit
- Amplification: GlobalFiler™ Amplification Kit
- Capillary Electrophoresis: 3500xL

STARlet used for
- Extraction
- Quantification plate setup
- Normalization
- Amplification plate setup

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Results

Two criteria were used to evaluate the validation data:

Male DNA Recovery
- assessed using quantification data
  - [human DNA] / [male DNA] in sperm and non-sperm fractions
  - % male DNA recovery in sperm and non-sperm fractions

Male DNA STR Profiles
- assessed using capillary electrophoresis data
  - determined if profiles are distinguishable (single source, major, minor, or deduced foreign)

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Results

Overnight Incubation of Lysates

- timeframes
  - no overnight incubation – same day extraction
  - **1 night incubation**
  - 3 nights incubation
- samples
  - varying sample type (serial dilution, proficiency tests, post-coital)
  - same AutoLys run
- no major difference between the timeframes
  - [male DNA] in sperm fraction samples slightly higher in 3-night incubation samples

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Overnight Incubation

	0-night incubation				1-night incubation				3-night incubation			
	Sperm Fraction		Non-Sperm Fraction		Sperm Fraction		Non-Sperm Fraction		Sperm Fraction		Non-Sperm Fraction	
	human DNA (ng/µL)	male DNA (ng/µL)	human DNA (ng/µL)	male DNA (ng/µL)	human DNA (ng/µL)	male DNA (ng/µL)	human DNA (ng/µL)	male DNA (ng/µL)	human DNA (ng/µL)	male DNA (ng/µL)	human DNA (ng/µL)	male DNA (ng/µL)
PC vaginal swabs (0 hours)	2.474	4.455	13.886	1.168	1.427	2.812	12.596	1.366	2.876	4.531	11.423	1.031
PC vaginal swabs (8 hours)	7.252	0.293	17.790	0.005	7.077	0.302	29.334	0.006	11.117	0.312	19.646	0.006
PC vaginal swabs (16 hours)	1.076	0.002	17.104	---	2.205	0.005	31.798	---	5.595	0.007	25.240	0.001
PC vaginal swabs (24 hours)	9.346	0.002	25.347	---	10.402	0.007	34.696	---	2.658	0.002	12.586	---

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Results

AutoLys-SpermX to QIAcube Comparison

- QIAcube
  - higher % male DNA recovery in sperm fractions of samples with lower levels of SF
- AutoLys-SpermX
  - higher % of carry-over (from non-sperm fraction to sperm fraction)
  - higher [male DNA] in sperm fractions

Male STR DNA Profiles

- distinguishable profiles (single source, major, minor, or deduced foreign)
  - determined by calculating Genotype Mixture Ratio (GMR)
  - STR profiles classified in 3 categories (single source vs. GMR > 3:1 vs. GMR < 3:1)

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Results

			AutoLys-SpermX					QIAcubes			
			Sperm Fraction		Non-Sperm Fraction			Sperm Fraction		Non-Sperm Fraction	
	Sample Type		human DNA (ng/uL)	male DNA (ng/uL)	human DNA (ng/uL)	male DNA (ng/uL)		human DNA (ng/uL)	male DNA (ng/uL)	human DNA (ng/uL)	male DNA (ng/uL)
Serial Dilution (buccal + SF)	Buccal swab + 1:1 SF*		21.182	19.055	4.217	0.102		14.999	15.282	5.657	0.073
		~ % male DNA	89.958		2.427		~ % male DNA	101.884		1.294	
	Buccal swab + 1:10 SF*		5.002	1.984	8.833	0.012		0.638	0.655	9.327	0.006
		~ % male DNA	39.674		0.135		~ % male DNA	102.565		0.060	
	Buccal swab + 1:50 SF*		2.124	0.237	5.655	0.002		0.058	0.065	7.856	0.001
		~ % male DNA	11.159		0.029		~ % male DNA	111.569		0.008	
	Buccal swab + 1:100 SF*		3.220	0.215	12.011	0.002		0.135	0.123	9.467	0.002
		~ % male DNA	6.666		0.020		~ % male DNA	91.152		0.021	
	Buccal swab + 1:500 SF*		0.550	0.030	5.673	0.0002		0.017	0.007	7.750	0.0002
		~ % male DNA	5.476		0.004		~ % male DNA	39.669		0.003	
	Buccal swab + 1:1000 SF*		1.701	0.002	8.759	---		0.007	0.0002	7.593	
		~ % male DNA	0.111		N/A		~ % male DNA	3.390		N/A	

*Samples run in triplicate for AutoLys-SpermX method and in duplicate for QIAcube method (average of results are represented in this table)

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Results

			AutoLys-SpermX					QIAcubes			
			Sperm Fraction		Non-Sperm Fraction			Sperm Fraction		Non-Sperm Fraction	
	Sample Type		human DNA (ng/uL)	male DNA (ng/uL)	human DNA (ng/uL)	male DNA (ng/uL)		human DNA (ng/uL)	male DNA (ng/uL)	human DNA (ng/uL)	male DNA (ng/uL)
Proficiency Tests	Fabric w/HB and SF (2018)*		1.414	1.388	0.071	0.009		0.135	0.165	0.186	0.021
		~ % male DNA	121.673		12.535		~ % male DNA	122.365		11.050	
	Fabric w/HB and SF (2019)*		0.047	0.052	0.077	0.004		0.309	0.350	0.231	0.010
		~ % male DNA	110.995		5.209		~ % male DNA	113.224		4.229	
	Fabric w/HB and SF (2020)*		0.688	0.969	0.096	0.071		0.205	0.279	0.167	0.075
		~ % male DNA	140.703		74.074		~ % male DNA	135.989		45.060	

*Samples run in duplicate for AutoLys-SpermX method (average of results are represented in this table)

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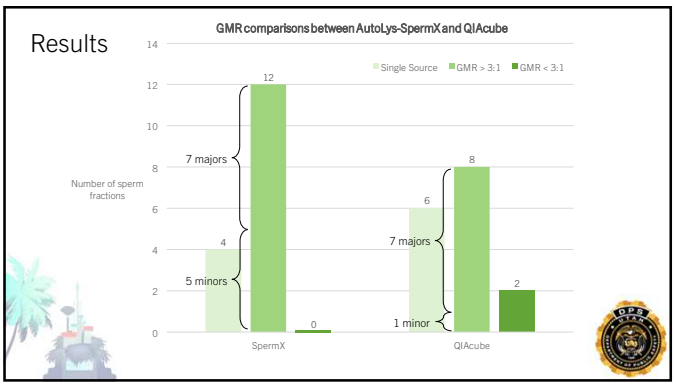
Results



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Conclusions

QIAcube Pathway
- run time for differential separation is ~7hrs for 36 samples
  - hands-on time is ~5.3hrs (~12hrs for 96 samples)
- less carryover of non-sperm fraction into sperm fraction
  - reason? – substrates remain in SpermX tubes

AutoLys-SpermX Pathway
- run time for differential separation is ~13.5hrs for 96 samples
  - hands-on time is ~4hrs
- higher [male DNA] in sperm fractions
- [male DNA] in sperm fraction samples slightly higher in 3-night incubation samples

Comparable % Male Recovery (in samples with less carryover between fractions)
- especially clear with proficiency test samples

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Conclusions

AutoLys-SpermX Method
- increases throughput
  - effective for large-scale processing of sexual assault samples
- increases time efficiency
  - frees up analyst time to perform other, more complex, tasks
- maintains individual sample integrity
- can be easily implemented in any laboratory setting

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Considerations

Thoughts for implementation
- downstream extraction capabilities
- amenable to different automated platforms, manual processes, or chemistries
- InnoGenomics provides reagents with their kits
  - newest buffer formulation reduces female carryover
  - determine if in-house buffer optimization is needed
- one deck layout for all sample types and methods

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