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

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
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 (AutoLysSpermX)
  - 96 sperm fractions, 96 non-sperm fractions

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
**Validation Setup**

**Pre-validation Optimization**
- 10mg/mL ProK vs. 20mg/uL ProK + different ratios (epithelial digestion)
- 1 epithelial digestion vs. 2 epithelial digestions
- 0.2 buffer vs. 04 vs. sterile water (sperm washes)

**Sample Preparation**
- post-coital samples, proficiency tests, casework-type samples, 5F serial dilutions, male mixtures, 5F 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

**Validation Workflow**

**Fully-Automated Method (new)**
1st epithelial digestion for 1.5hrs
Lift-and-Lock + centrifuge
( Substrates remain within inner column of SpermX tubes)
96 non-sperm fractions removed (ready for extraction)
new sample tubes loaded for sperm fractions
2nd epithelial digestion for 30m
Lift-and-Lock + centrifuge (lysate discarded)
Wash steps performed 3x for sperm cells remaining in SpermX tubes
Sperm digestion for 45m
Lift-and-Lock + centrifuge
96 sperm fractions removed (ready for extraction)

**Semi-Automated Method (current)**
1st epithelial digestion for 1.5hrs
Substrates removed manually utilizing spin baskets
(12 samples each for a total of 144 samples)
96 sperm fractions removed for manual addition of buffer + incubation (additional tips and sperm digest buffer are loaded)
Final wash steps performed for sperm cells within QIAcubes for total of 4 washes
36 sperm fractions removed for manual addition of buffer
Sperm digestion for 30m
72 non-sperm + sperm fractions ready for extraction

**UBFS AutoLys-SpermX Deck Layout**

[Image of UBFS AutoLys-SpermX Deck Layout]
UBFS AutoLys-SpermX Deck Layout

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

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)
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

Overnight Incubation

<table>
<thead>
<tr>
<th></th>
<th>Sperm Fraction</th>
<th>Non-Sperm Fraction</th>
<th>Sperm Fraction</th>
<th>Non-Sperm Fraction</th>
<th>Sperm Fraction</th>
<th>Non-Sperm Fraction</th>
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</thead>
<tbody>
<tr>
<td><strong>human DNA</strong></td>
<td>2.474</td>
<td>4.455</td>
<td>13.886</td>
<td>1.168</td>
<td>1.427</td>
<td>2.812</td>
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<tr>
<td><strong>male DNA</strong></td>
<td>12.596</td>
<td>1.366</td>
<td>11.423</td>
<td>1.031</td>
<td>2.879</td>
<td>3.500</td>
</tr>
</tbody>
</table>

Overnight Incubation

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<thead>
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<th>Sperm Fraction</th>
<th>Non-Sperm Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>human DNA</strong></td>
<td>7.252</td>
<td>0.293</td>
<td>17.790</td>
<td>0.005</td>
<td>7.077</td>
<td>0.302</td>
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<tr>
<td><strong>male DNA</strong></td>
<td>29.334</td>
<td>0.006</td>
<td>19.646</td>
<td>0.006</td>
<td>11.117</td>
<td>0.006</td>
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</tbody>
</table>

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)
### Results

#### AutoLys-SpermX vs. QIAcubes

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Sperm Fraction</th>
<th>Non-Sperm Fraction</th>
<th>Sperm Fraction</th>
<th>Non-Sperm Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal swab</td>
<td>10.05</td>
<td>0.20</td>
<td>10.00</td>
<td>0.20</td>
</tr>
<tr>
<td>Buccal swab + 1:1</td>
<td>21.18</td>
<td>3.24</td>
<td>21.05</td>
<td>3.24</td>
</tr>
<tr>
<td>Buccal swab + 1:10</td>
<td>5.00</td>
<td>2.98</td>
<td>5.00</td>
<td>2.98</td>
</tr>
<tr>
<td>Buccal swab + 1:50</td>
<td>2.12</td>
<td>0.50</td>
<td>2.12</td>
<td>0.50</td>
</tr>
<tr>
<td>Buccal swab + 1:100</td>
<td>3.22</td>
<td>0.43</td>
<td>3.22</td>
<td>0.43</td>
</tr>
</tbody>
</table>

#### Serial Dilution (buccal + SF)

- **Buccal swab + 1:1 SF**
  - Human DNA: 21.182 ng/μL, Male DNA: 19.055 ng/μL
  - % male DNA: 89.958
- **Buccal swab + 1:10 SF**
  - Human DNA: 5.002 ng/μL, Male DNA: 1.984 ng/μL
  - % male DNA: 39.674
- **Buccal swab + 1:50 SF**
  - Human DNA: 2.124 ng/μL, Male DNA: 0.237 ng/μL
  - % male DNA: 11.159
- **Buccal swab + 1:100 SF**
  - Human DNA: 3.220 ng/μL, Male DNA: 0.215 ng/μL
  - % male DNA: 6.666
- **Buccal swab + 1:500 SF**
  - Human DNA: 0.550 ng/μL, Male DNA: 0.030 ng/μL
  - % male DNA: 5.476
- **Buccal swab + 1:1000 SF**
  - Human DNA: 1.701 ng/μL, Male DNA: 0.002 ng/μL
  - % male DNA: 1.111

#### Proficiency Tests

- **Fabric w/HB and SF (2018)**
  - Human DNA: 1.414 ng/μL, Male DNA: 1.388 ng/μL
  - % male DNA: 121.673
- **Fabric w/HB and SF (2019)**
  - Human DNA: 0.047 ng/μL, Male DNA: 0.052 ng/μL
  - % male DNA: 110.995
- **Fabric w/HB and SF (2020)**
  - Human DNA: 0.688 ng/μL, Male DNA: 0.969 ng/μL
  - % male DNA: 140.703

#### GMR comparisons between AutoLys-SpermX and QIAcubes

- **Single Source**
  - GMR > 3:1: 7 majors
  - GMR < 3:1: 5 minors

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

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

Considerations

Thoughts for implementation
- Downstream extraction capabilities
- Amenable to different automated platforms, manual processes, or chemistries
- InnoGenomics provides reagents with their kits
- Newer 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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