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AN ANALYSIS OF EXTRACTION EFFICIENCIES OF VARIOUS SWABS ON SPERM RECOVERY

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Introduction

With the advancements in the field of biology, aspects of sexual assault kits and the way they are processed have been improved. These improvements relate to the differential extraction process and the technological advancements that allow mixtures to be interpreted. However, there is one element of these sexual assault examination kits that has remained constant over time; the cotton swab used as the collection device. Despite the research done suggesting the cotton swab's absorbent nature and its inclination to retain cellular material, no implementation of another swab has been made into the field of forensic nursing. Research has shown that other swabs, such as the nylon flocked swab, have out-performed the cotton swab when testing for collection and elution efficiencies. When samples are being taken for DNA testing it is important that the collection device is as efficient at collecting cellular material as it is at eluting cellular material when an extraction is done. Medical collection devices also have strong research showing their efficiency at collecting and eluting cellular material, especially the cytobrush, used for gynecological purposes. This study aims to address the research gap of determining if the cotton swab is an efficient enough collection device for continued use in sexual assault examination kits.

Swabs Studied



Cotton



Nylon Flocked



Cytobrush

Research Question

- Does the swab type used in sexual assault examination collection have a role in how well cellular material is released from the collection device

Goals of Research

- Optimize a collection device for sexual assault examinations
- Determine which swab has the highest extraction efficiency, which could be beneficial for sexual assault investigations
- Determine if swab structure has an impact on elution of male DNA

Hypothesis

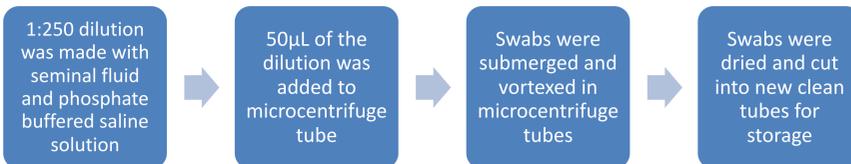
H₀: There will not be any difference in swab efficiencies for sperm recovery

H_A: The nylon flocked and cytobrush swabs will be more efficient than the cotton swab for sperm recovery, and the cytobrush swab will be the most efficient for sperm recovery

Methods

Sample Preparation

- Samples were prepared with seminal fluid obtained from previous research and purchased from BioIVT
- There were twelve seminal fluid samples used in this study (labeled MS1-12)
- Preparation was based off mock sexual assault sample preparation done at the Allegheny County Medical Examiners Office



Methods Continued

- Cotton, nylon, and cytobrush swabs were all prepared in triplicate for each seminal fluid sample (3 swab types per seminal fluid sample)
- 108 samples were made in total, 9 swabs total per seminal fluid sample

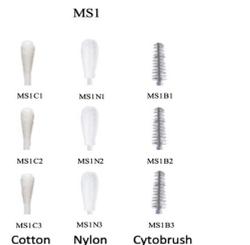


Figure 1: Samples Prepared for Seminal Fluid Sample 1 (MS1)



Figure 2: Cytobrush Swabs Drying After Preparation



Figure 3: Cotton Swabs Drying After Preparation

Microcentrifuge tubes after samples were prepared:



Figure 4: Microcentrifuge Tube After Preparation (Cotton)



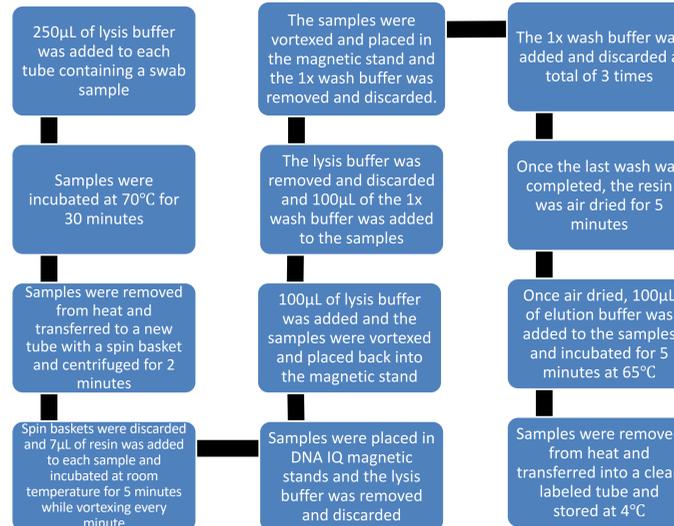
Figure 5: Microcentrifuge Tube After Preparation (Nylon)



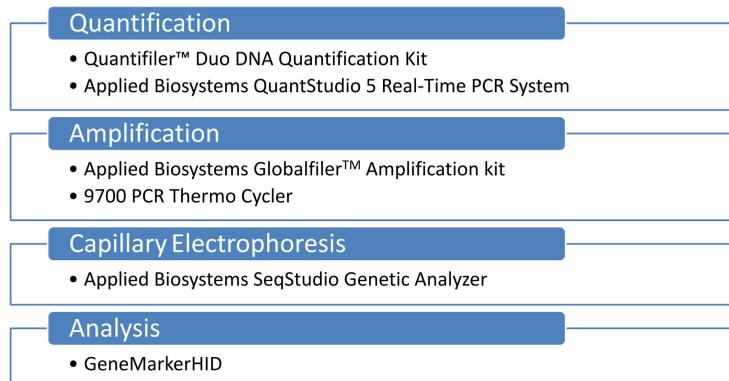
Figure 6: Microcentrifuge Tube After Preparation (Cytobrush)

Extraction Procedure

- The DNA IQ System—Database, DNA Isolation from Stains and Buccal Swabs protocol was used
- One optimization was made for this protocol
 - The concentration of DTT added to the lysis buffer was increased to 10 times the suggested amount
 - 10µL DTT per 100µL lysis solution



- Each swab type was also prepared as a negative control and an FTA card deposited with blood was prepared as a positive control



Results

COMPARISON OF AVERAGE QUANTIFICATION VALUES BETWEEN SWAB TYPES

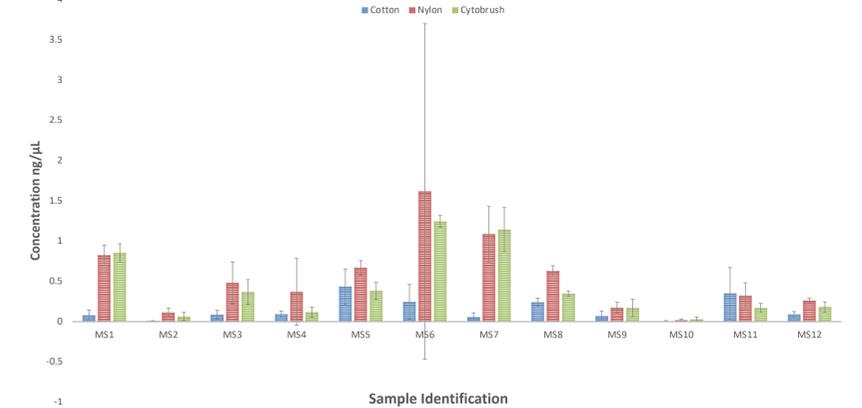


Table 1: Statistical Analysis to Determine Significance Differences Between Swab Types

Swabs Compared	Calculated T-test Value of Samples												Significantly Different
	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8	MS9	MS10	MS11	MS12	
Cotton & Nylon	9.23795	3.23471	2.57787	1.15659	1.69850	1.13257	5.11395	8.12337	1.96179	1.10062	0.13870	6.25621	P value 0.05 DOF 4 Critical T-Value 2.776
Cotton & Cytobrush	10.40154	1.81495	2.96805	0.54866	0.36267	7.65248	6.74890	3.08491	1.40774	1.13718	0.95851	2.15969	
Nylon & Cytobrush	0.29013	1.13061	0.64726	1.04907	3.58355	0.30803	0.22717	6.70002	0.02322	0.50546	1.53481	1.90735	

Discussion/Conclusions

- The nylon flocked and cytobrush swabs showed that 41.667% of both the nylon flocked and cytobrush samples were significantly different than the cotton swab samples.
- When comparing the nylon flocked swabs to the cytobrush swabs to determine if the sperm concentration values were significantly different, two nylon flocked and cytobrush swabs had t-values larger than the critical t-value to show that they were significantly different (Table 1).
- After cytobrush swabs were prepared there was fluid sample left in the microcentrifuge tube (Figure 6), contrary to the cotton swab tube after preparation (Figure 4).
- It is important to note that the cytobrush concentration values were higher than cotton concentration values in 41.667% of samples even though the cytobrush did not absorb the sample completely while being prepared. This supports the research suggesting that the cotton swab does not elute material well because of its inner absorbent matrix.
- The cytobrush and its open structure suggests that swab structure influences elution.
- The nylon flocked swab also left trace amounts of fluid behind at the initial sample preparation step (Figure 5). The nylon flocked swab designed to keep cellular material at the surface of the swab, also showed that swab structure has an impact on cellular elution. This swab type also had 41.667% of samples where the sperm concentrations were higher and significantly different from the cotton swab concentrations.
- Sample MS6, MS7, and MS8 will be taken on to genotyping to determine differences in peak height and overall quality of electropherogram for the three different swab types.
- More research needs to be done to provide support for these conclusions.

Future Directions

- Include female DNA and use a differential extraction to make this study as realistic to sexual assault case work as possible
- Use cervical nylon flocked swabs
- Run data in sets of five, instead of three
- Test other swabs used in the medical field
- Include time parameters to see if it affects absorption factors
- Extract and re-extract swabs to determine if more DNA can be obtained from a pre-extracted swab

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