2021-03-08

An Analysis of Extraction Efficiencies of Various Swabs on Sperm Recovery

Lindsey Campany

AN ANALYSIS OF EXTRACTION EFFICIENCIES OF VARIOUS SWABS ON SPERM RECOVERY

Lindsey Campany, B.A.*, Pamela Marshall, Ph.D., Lisa Ludvico, Ph.D., Elizabeth Wisbon, M.S.
Forensic Science & Law Program Duquesne University, Pittsburgh, PA, 15282, USA

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

H_0: 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 pervious 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

1:250 dilution was made with seminal fluid and phosphate buffered saline solution → 50µL of the dilution was added to microcentrifuge tube → Swabs were submerged and vortexed in microcentrifuge tubes → Swabs were dried and cut into new clean tubes for storage

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)

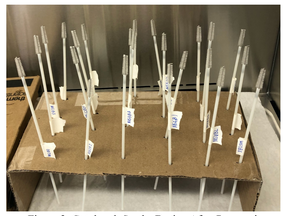

Figure 2: Cytobrush Swabs Drying After Preparation

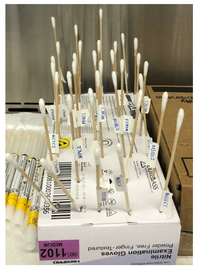

Figure 3: Cotton Swabs Drying After Preparation

Microcentrifuge tubes after samples were prepared:

Cotton

Figure 4: Microcentrifuge Tube After Preparation (Cotton)

Nylon Flocked

Figure 5: Microcentrifuge Tube After Preparation (Nylon)

Cytobrush

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

250µL of lysis buffer was added to each tube containing a swab sample → Samples were incubated at 70°C for 30 minutes → Samples were removed from heat and transferred to a new tube with a spin basket and centrifuged for 2 minutes → Spin baskets were discarded and 7µL of resin was added to each sample and incubated at room temperature for 5 minutes while vortexing every minute → Samples were placed in DNA IQ magnetic stands and the lysis buffer was removed and discarded → 100µL of lysis buffer was added and the samples were vortexed and placed back into the magnetic stand → The lysis buffer was removed and discarded and 100µL of the 1x wash buffer was added to the samples → The samples were vortexed and placed in the magnetic stand and the 1x wash buffer was removed and discarded. → The 1x wash buffer was added and discarded a total of 3 times → Once the last wash was completed, the resin was air dried for 5 minutes → Once air dried, 100µL of elution buffer was added to the samples and incubated for 5 minutes at 65°C → Samples were removed from heat and transferred into a clean labeled tube and stored at 4°C

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

Quantification
- Quantifiler™ Duo DNA Quantification Kit
- Applied Biosystems QuantStudio 5 Real-Time PCR System

Amplification
- Applied Biosystems Globalfiler™ Amplification kit
- 9700 PCR Thermo Cycler

Capillary Electrophoresis
- Applied Biosystems SeqStudio Genetic Analyzer

Analysis
- GeneMarkerHID

Results

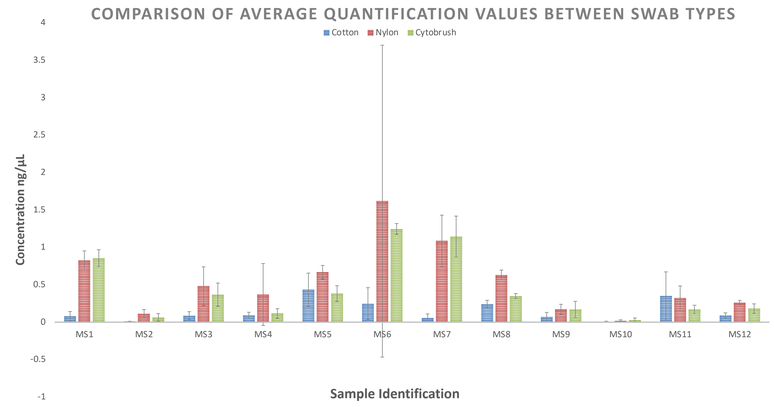

Table 1: Statistical Analysis to Determine Significance Differences Between Swab Types

Calculated T-test Value of Samples												
Swabs Compared	**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
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

Significantly Different

Statistical Parameters	
P value	0.05
DOF	4
Critical T-Value	2.776

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

Acknowledgements

- Bayer School of Natural and Environmental Science
- Forensic Science and Law Program
- Pamela Marshall, Ph.D., Duquesne University University
- Lisa Ludvico, Ph.D., Duquesne University
- Elizabeth Wisbon, M.S., Allegheny County Medical Examiner's Office
- Forensic Science and Law Class of 2021

Contact Information

Email: Campanyl@duq.edu

References

- Butler, J. M., Chapter 2 - DNA Extraction Methods. In *Advanced Topics in Forensic DNA Typing: Methodology*, Butler, J. M., Ed. Academic Press: San Diego, 2012; pp 29-47.
- Benschop, C. C. G.; Wiebosch, D. C.; Kloosterman, A. D.; Sijen, T., Post-coital vaginal sampling with nylon flocked swabs improves DNA typing. *Forensic Science International: Genetics* **2010,** *4* (2), 115-121.
- Brownlow, R. J.; Dagnall, K. E.; Ames, C. E., A Comparison of DNA Collection and Retrieval from Two Swab Types (Cotton and Nylon Flocked Swab) when Processed Using Three QIAGEN Extraction Methods. *Journal of Forensic Sciences* **2012,** *57* (3), 713-717.
- "DNA IQ™ System—Database Protocol". *Promega.Com*, 2016. Accessed 22 Nov 2020.