Cotton Swabs vs. 4N6FLOQSwabs™: A Comparative Study for Optimal Recovery of Simulated Touch DNA

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2013
Washington, D.C.

INTRODUCTION

In forensic laboratories moistened cotton swabs are often used to collect DNA evidence. These swabs are made of cotton fibers tightly wrapped around the tip of a wooden stick. While highly absorbent, the dense inner core can trap cellular materials within its fibers. An alternative type of swab called 4N6FLOQSwabs™ (Copan Italia, Brescia, Italy) are instead made of thousands of parallel short nylon strands that are flocked onto a plastic stick (images 1 - 4). Due to this unique feature, these swabs lack an inner core that can trap cellular materials. This becomes especially important when working with low-level or touch DNA, as a dense core may trap the few cells present. The first objective of this study was to compare the cell/DNA recovery obtained with cotton and nylon flocked swabs from samples placed on various substrates.

The manufacturers of the nylon flocked swab has also developed a specialized spin basket called a Nucleic Acid Optimizer (NAO) that has been found to increase recovery of nucleic acids by 60% (1). The NAO consists of a semi-permeable basket, which retains fluid until placed in a centrifuge. The second objective of this study was to determine the effect of the NAO on nucleic acid recovery.

Flocked vs cotton swab recovery was evaluated with two different extraction kits PrepFiler® Forensic DNA Extraction Kit (Life Technologies) and DNA IQ™ System (Promega) on multiple substrates shown to affect DNA recovery (2).

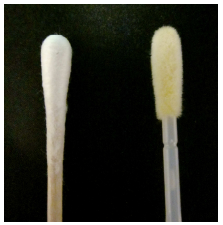

Image 1: A traditional cotton swab (left) and a nylon flocked swab (right).

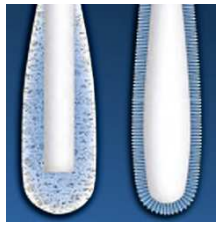

Image 2: Illustrated cross sections of cotton (left) and nylon flocked (right) swabs.

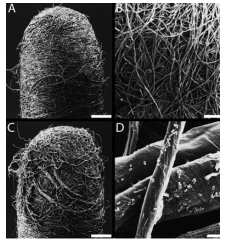

Image 3: SEM images of cotton swab.

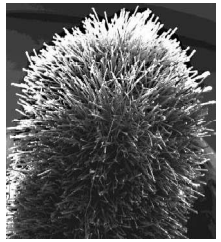

Image 4: SEM image of nylon flocked swab.

MATERIALS & METHODS

DNA Samples

Lymphocytes were isolated from fresh blood sample using Histopaque® 1077 (Sigma-Aldrich, St. Louis, MO). Cell count was determined by using a hemocytometer and a dilution of ~80 cells/uL was prepared using PBS as a dilutent.

Substrates

25uL aliquots of the cell solution were then placed on the following substrates and allowed to dry:

-Glass Slides -Metal Knife Handle -Plastic
-Leather Belt -Wood (Unfinished) -Plastic Gun Grips

Sample Collection

Collection was performed by wetting half of the swab with 20µL of 0.01% SDS and using a wet-dry swabbing technique. The experiment was performed in double triplicates.

Immediately after collection, swab heads were removed by inserting the swab in the tube and simply bending the shaft at the pre-molded 20mm breaking point (right); nylon flocked swab heads were broken off at the break point on the handle while the cotton swab heads were removed by shaving the cotton from the wood shaft. Swabs were placed into 2mL tubes. Half were then extracted with NAO and half without.

Image 5: NAO and Flocked Swab broken in NAO

Sample Extraction

PrepFiler® was used as recommended by the manufacturer. Flocked swab samples extracted with the *DNA IQ™ System* showed extraction inhibition when the swab was incubated in the DNA IQ™ Lysis Buffer for one hour. Other authors have reported denim dyes and Hemastix® reagents, among other things, to interfere with DNA binding to the DNA IQ™ magnetic beads greatly decreasing DNA yield (3).

To overcome this issue, the protocol was modified as follows for both swabs types: once swabs were in tube/NAO and Lysis Buffer was added, all samples were vortexed at maximum speed for 30 seconds and centrifuged for 2 minutes at 14000 rpm. Swabs or NAOs containing swabs were removed from their tubes and discarded. The filtrate was then incubated according to the recommended protocol, which was followed from this point on.

Quantitation

Quantifiler® Human DNA Quantification Kit was used as recommended by the manufacturer. Quantification was performed on an ABI PRISM® 7000 Sequence Detection System.

RESULTS

A) Nylon Flocked Swab vs Cotton Swab Recovery with DNA IQ™ (NAO Used)

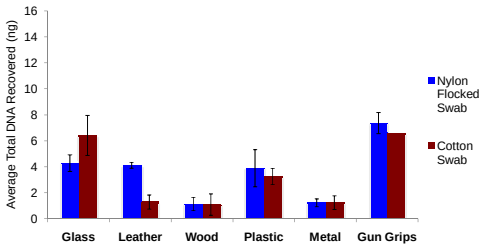

Figure A. Swabs performed similarly with DNA IQ™ and NAO.

B) Nylon Flocked Swab vs Cotton Swab Recovery with PrepFiler® (NAO Used)

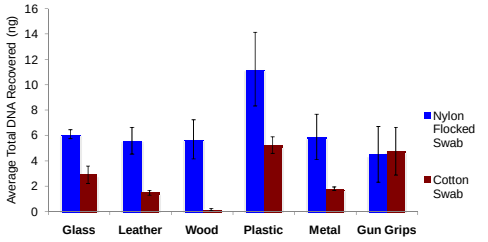

Figure B. Nylon flocked swab performed better on 5 of the 6 substrates with PrepFiler® and NAO.

C) Nylon Flocked Swab vs. Cotton Swab Recovery with DNA IQ™ (No NAO Used)

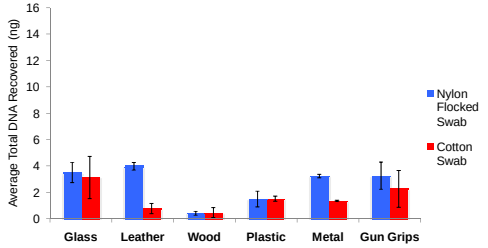

Figure C. Swabs performed similarly with DNA IQ™ and no NAO.

D) Nylon Flocked Swab vs. Cotton Swab Recovery with PrepFiler® (No NAO Used)

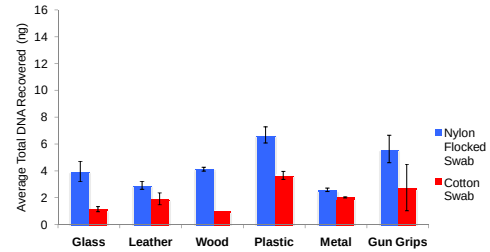

Figure D. Nylon flocked swabs performed better on all substrates with PrepFiler® and no NAO.

DISCUSSION

- Both types of swabs performed similarly when extracted using DNA IQ™.
- Flocked swabs yielded greater amounts of DNA then cotton swabs when extracted with PrepFiler®.
- On average samples collected from a substrate and processed using an NAO had a 34% higher DNA yield across all substrates and kits than those processed without a NAO.
- DNA yield when collected with nylon flocked swabs produced more consistent results across substrates when extracted with PrepFiler®.
- The source of the incompatibility between the nylon flocked swab and the DNA IQ™ Lysis Buffer remains unknown due to the proprietary nature of the products. A modified protocol to overcome the issue was successfully developed by removing the flocked swab prior to the incubation step.

CONCLUSIONS

- In our experiment, best recoveries occurred when a sample was collected with a nylon flocked swab, processed with an NAO, and extracted with PrepFiler®.
- It is imperative to modify the extraction protocol if processing nylon flocked swabs with DNA IQ™ (i.e. avoid incubation of the swab in the lysis buffer).

ACKNOWLEDGMENTS

We would like to thank Copan Italia, Life Technologies, Dr. Moses Schanfield, and Lori Prugh for their support and advice on this project.

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