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

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Introduction

In forensic laboratories, moistened cotton swabs are often used to collect DNA evidence, but the dense inner core can trap cellular materials within its fibers (Image 1). An alternative type of swab, 4N6FLOQSwabs™ (Copan Italia, Brescia, Italy), is made of parallel short nylon strands that are flocked onto a plastic stick, which lack an inner core (Image 1).

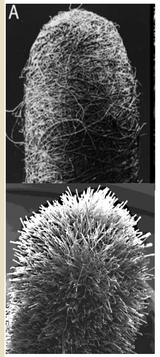


Image 1: SEM images of cotton swab (top) and nylon swab (bottom).

This study was to compare DNA recovery of cotton and nylon flocked swabs from lymphocyte samples collected from various substrates, which have been shown to affect DNA yield, and to determine the best extraction method (1). A specialized spin basket, the Nucleic Acid Optimizer (NAO™), has also been developed. It is a semi-permeable basket that retains fluid until centrifuged, reducing the number of sample transfer steps reducing chances of contamination (2). The other objective of this study was to determine the effect of the NAO™ on nucleic acid recovery.

Due to its design, it has been proposed that 4N6FLOQSwabs™ are also more effective at releasing cellular materials than cotton swabs. Thus, DNA release from blood samples on nylon flocked and cotton swabs was also performed.

The 4N6FLOQSwabs™ are packaged and stored in sterile sample collector tubes and are treated with an antimicrobial agent (Image 2) . Another study was conducted to evaluate the antimicrobial activity of 4N6FLOQSwabs™.

Image 2: Antimicrobial-treated 4N6FS swab with sample collector tube

Blood Collection Study

Sample Preparation and Extraction

Blood was collected and stored at -20˚C with EDTA. Aliquots of 5 µL were spotted on glass in six replicates per condition. Stains were allowed to dry for 24 hours and collected with nylon flocked swabs and cotton swabs using water and the wet/dry technique. Aliquots were also spotted directly on nylon flocked swabs, cotton swabs, and in the tube. After two weeks, PrepFiler® was used to extract the samples following the protocol recommended by the manufacturer.

Results

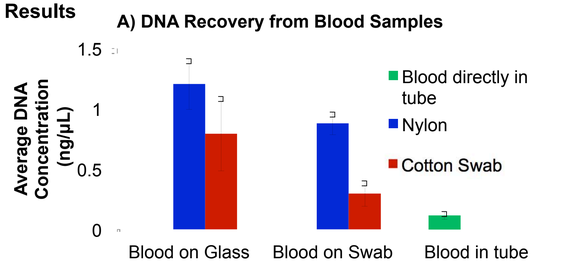


Figure A: Nylon swabs had higher recovery than the cotton swabs. The reduced DNA recovery from blood samples spotted directly in the tube is unexplained (see discussion).

Lymphocyte Collection Study

Samples and Substrates

Lymphocytes were isolated from fresh blood using Histopaque® 1077 (Sigma-Aldrich, St. Louis, MO). 25 µL of ~80 cells/µL dilution were spotted, dried, and collected from the following substrates: Glass slides, Plastic, metal knife handle, leather belt, unfinished wood, plastic gun grips.

Sample Collection

Wet- dry swabbing was performed for each swab with 20 µL of 0.01% SDS. Nylon flocked swab heads were broken off at the break point, while the cotton swab heads were shaved off.

Extraction & Quantitation

Half of the samples were extracted with the NAO™ and half were extracted without. PrepFiler® was used as recommended by the manufacturer. Flocked swab samples extracted with the *DNA IQ™ System* showed extraction inhibition in the DNA IQ™ Lysis Buffer. The protocol was modified: Lysis Buffer was added to swabs in the tube/NAO™, then samples were vortexed at maximum speed for 30 seconds and centrifuged for 2 minutes at 14,000 rpm. The filtrate was then incubated according to the recommended protocol, which was followed from this point on.

In all of the studies, samples were quantified using the Quantifiler® Human DNA Quantification Kit.

Results

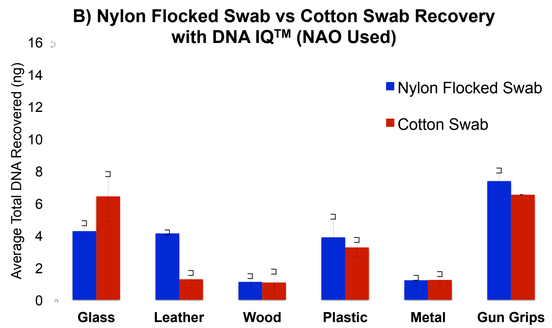


Figure B. Swabs performed similarly with DNA IQ™ and the NAO.

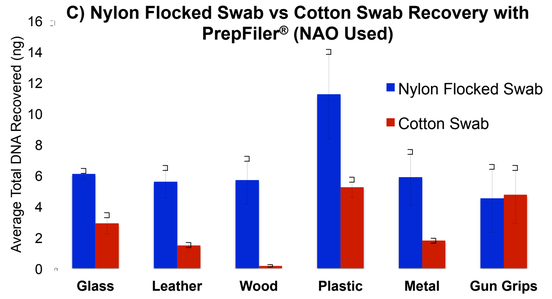


Figure C. Nylon flocked swab performed better overall with PrepFiler® and the NAO.

On average, samples collected from a substrate and processed using an NAO had a ***48%*** higher DNA yield across all substrates, swabs, and kits than those processed without a NAO™.

Bacterial Contamination Study

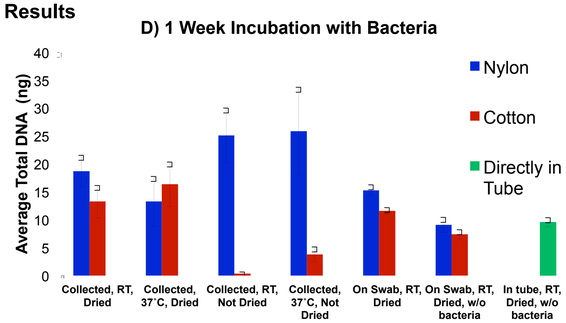
Bacterial cell suspension

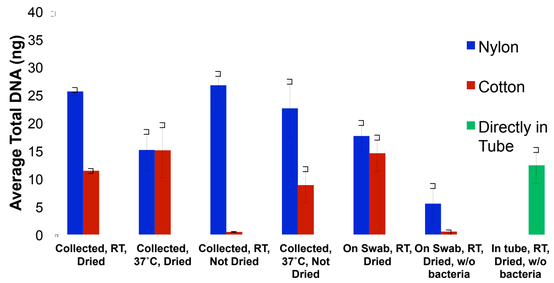
Bacteria were collected by swabbing fingers, an office door knob, a restroom door knob, and a cheek swab. Cells were grown on agar plates, and colonies were picked from each plate and grown in solution of LB broth.

Sample Preparation and Extraction.

Cells were collected from 10 buccal swabs and were isolated in 10 mL of 1X PBS. Twenty (20) µL of buccal cell solution was spotted on plastic and allowed to dry. Samples were collected using wet/dry technique wetted with bacterial suspension (20 µL before and 20 µL after collection). Samples were also spotted directly onto nylon and cotton swabs with 20 µL of buccal solution, with and without bacteria, and directly into 1.5 mL tubes with no bacteria. All conditions were tested in triplicate. PrepFiler® with the NAO™ was used to extract the samples after 1 week and 2 weeks.

Results





Figures D and E. Nylon swabs performed better overall in each condition for both weeks. Results were consistent from one week to two weeks.

Statistical analysis, specifically factorial analysis of variance with repeated cases, was performed with a software called Statistica. Results show strong significance, p=0.00933, for the increased recovery of DNA with nylon flocked swabs vs cotton swabs spotted with bacteria and not allowed to dry.
Furthermore, it was surprising to find that the samples that were spotted directly on the swab and in the tube without bacteria yielded lower DNA quantities than the samples that were spotted with bacteria. Preliminary experimental data (not shown) suggest that bacterial DNA is acting as a carrier for human DNA during the extraction process. Further experiments to confirm this assumption are ongoing.

Discussion

In our hands nylon flocked swabs provided better DNA recovery from bloodstains. The increase DNA recovery with nylon swabs, when blood was spiked directly onto the swab, suggest that flocked swabs are more effective at releasing cellular materials from the inner core compared to cotton swabs.

In the lymphocyte study, nylon flocked swabs produced more consistent results across substrates when extracted with PrepFiler® compared to DNA IQ™. The NAO™ increased nucleic acid recovery with both swabs and extraction methods tested.

The source of the incompatibility between the nylon flocked swab and the DNA IQ™ Lysis Buffer remains unknown; yet a modified protocol to overcome the issue was successfully developed. However, it should be noted that this modified protocol could decrease nylon flocked swab yield when extracted with DNA IQ™.

In the bacterial study, the nuclease activity of the bacteria was confirmed by the low DNA recovery from cotton swabs that were not dried. DNA recovery from nylon flocked swabs with or without bacterial contamination was consistent whether the sample was dried or not dried. Furthermore, week 1 and week 2 showed consistent results, suggesting that most bacterial damage on human DNA occurs within the first week.

The reduced yield obtained from samples directly spotted in the tube (both blood and buccal cells) remains unexplained. In the bacterial study the increased yield obtained from samples with bacteria compared to samples without bacteria suggests that the bacterial DNA may act as a carrier. Results from preliminary experiments to test this theory (data not shown) support the assumption. Further experiments are currently being performed.

Conclusions

In our hands, across all the tested conditions, best DNA recoveries occurred when a sample was collected with a nylon flocked swab, processed with an NAO™, and extracted with PrepFiler® (Image 5).

Furthermore, the design of the nylon flocked swab, which lacks an inner core, allows for a better release of sample from the swab than the cotton swab (Image 6).

The antimicrobial activity of the 4N6FLOQSwabs™ was confirmed. Thus, after collection, nylon flocked swabs used at a crime scene can be immediately placed in the plastic sample tube, also providing a protected environment for the sample.

Image 5: Nylon flocked swab preparation in an NAO.

Image 6: Illustrated cross sections; Cotton (left) and Nylon (right).

References

1. Brownlow et al. *J Forensic Sci* 57(3):713-717 (2012).
2. Copan Flock Technologies website. Accessed 1 Aug 2013. http://www.copanflocktech.com/index.php/prod/sampleprep/nucleic/

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