4N6FLOQSWABS™, an Alternative for Sample Collection

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Abstract

The 4N6FLOQSwabs™ have been proposed as a more effective swab for releasing cellular materials than cotton swabs due to its nylon fibers and lack of an inner core. The recovery of DNA from 4N6FLOQSwabs™ samples can be increased with the use of the Nucleic Acid Optimizer (NAO™). 4N6FLOQSwabs™ also are treated with an antimicrobial agent for direct storage of swabs after collection to improve DNA yield.
Results showed that in our hands the 4N6FLOQSwabs™ provided statistically significant higher recovery of DNA than cotton swabs. Also, the antimicrobial activity of the 4N6FLOQSWABS™ was confirmed.
Thus, the 4N6FLOQSWABS™ can be considered as a valid alternative to the most commonly used cotton swab to collect DNA evidence at crime scenes and in forensic laboratories.

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). The swab is also designed to neutralize microbial contaminants while preserving the integrity of nucleic acids without the need to dry the swab.

Image 1: Illustrated cross sections; Cotton (left) and Nylon (right).

A specialized spin basket, the Nucleic Acid Optimizer (NAO™), has also been developed (Image 2). The NAO™ basket has four valves that retains fluid until centrifuged, reducing the number of sample transfer steps and chances of contamination (1).

Image 2: Nylon flocked swab preparation in an NAO™.

Previous studies have been conducted to compare the DNA recovery with cotton and nylon flocked swabs to determine the best extraction method and the effect of the NAO™ on nucleic acid recovery. PrepFiler® (Life Technologies™) was determined to be the best extraction method for the 4N6FLOQSwabs™, and the NAO™ showed increased nucleic acid recovery with both swabs and extraction methods tested.

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

The 4N6FLOQSwabs™ are packaged and stored in sterile sample collector tubes and are treated with an antimicrobial agent (Image 3) . Another study was conducted to evaluate the antimicrobial activity of 4N6FLOQSwabs™.

Image 3: Antimicrobial-treated 4N6FS swab with sample collector tube

Blood Collection Study

Sample Preparation and Extraction

Blood was collected and spotted directly for each type of sample. Five (5) µL of fresh blood were spotted in three replicates per sample type.
Blood was spotted on glass and allowed to dry then collected with nylon flocked swabs and cotton swabs using 20 µL of water and the wet/dry technique. Blood was also spotted directly onto nylon flocked swabs and cotton swabs. All cotton swabs were allowed to dry overnight prior to storage in manila envelopes. One set of nylon flocked swabs were allowed to dry overnight prior to storage in their corresponding collection tubes. Another set of nylon flocked swabs were not allowed to dry and placed directly into their corresponding collection tubes following collection. Blood was also spotted directly in the 2.0 mL tubes and allowed to dry overnight before storage as reference control. Samples were stored at room temperature for one week prior to extraction. PrepFiler® with the NAO™ was used to extract the samples following the protocol recommended by the manufacturer. Extracts were quantified using Quantifiler™ and the ABI PRISM® 7000.

Results

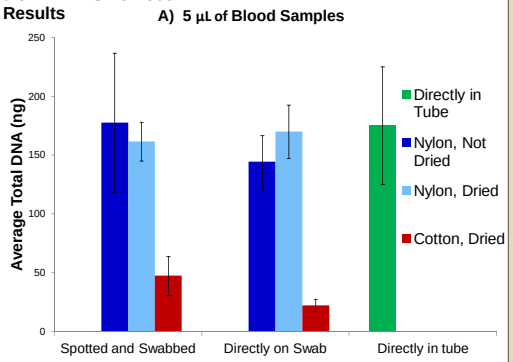


Figure A: Nylon swabs had higher recovery than the cotton swabs

Statistical analysis, specifically a set of T-tests, was performed with the software STATISTICA. Results showed that the increased recovery of DNA with nylon flocked swabs versus cotton swabs was highly significant, with a T-value of 9.021 and a p-value of 0.000. Furthermore, the difference in the recovery of cotton swabs to blood in the tube is highly significant, T=-6.474, p=0.000343. The recovery of DNA from nylon swabs that were dried before storage versus nylons swabs that were not dried was not significantly different. Lastly, the DNA yield in the nylon swabs was not significantly different than the yield from blood directly in the tube.

Similar experiments were previously performed to compare nylon swabs to cotton swabs with blood samples. In these experiments, DNA yield was significantly lower for blood spotted directly in the tube. It was determined that the process of drying the blood in the tube prior to extraction leads to higher DNA yields in comparison to extraction of wet blood samples, which was previously performed.

Bacterial Contamination Study

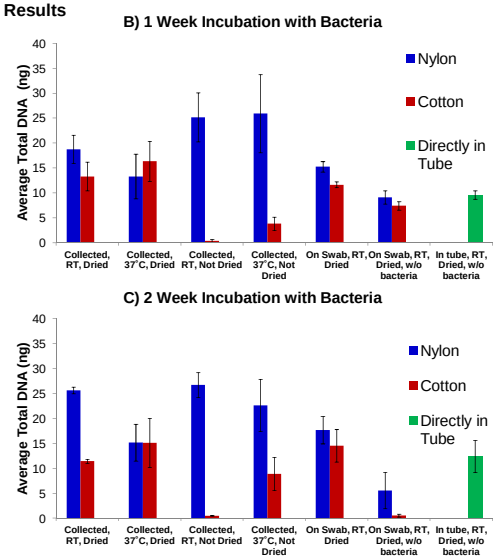
Bacterial cell suspension

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 from the same individual 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 on swabs 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 B and C. Nylon swabs performed better in each condition for both weeks overall. Results from one and two weeks were consistent.

Statistical analysis, specifically factorial analysis of variance with repeated cases, was performed with STATISTICA. Results showed that there is a strong significance, p=0.00933, for the increased recovery of DNA with nylon flocked swabs vs cotton swabs that are 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. Results (not shown) from recent experiments support the assertion that the bacterial DNA is acting as a carrier for the human DNA during the extraction process, leading to higher yield for samples that were incubated with bacteria.

Discussion

In the blood study, results showed that in our hands the nylon flocked swabs had equivalent recovery of DNA to that of the reference samples of blood directly in the tube. The cotton swabs, however, showed a significant loss in DNA yield when compared to recovery of blood in the tube.

Furthermore, the increase in the DNA recovery with nylon swabs, when blood was spiked directly onto the swab, when compared to the cotton swab shows that the nylon flocked swabs are more effective at releasing cellular materials from within the swab compared to cotton swabs.

Lastly, the blood study showed that there is not a significant difference in DNA yield whether the nylon swab is allowed to dry or placed directly in the collection tube, indicating that samples collected on the nylon swabs can be stored immediately after the sample is collected.

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.

Conclusions

The design of the nylon flocked swab, with fiber strands and lack of an inner core, allows for very efficient collection and release of DNA sample, resulting in the complete recovery of DNA. On the other hand, the cotton swab, which has a dense inner core , shows a significant loss of DNA (Images 1 and 4).

The antimicrobial activity of the 4N6FLOQSwabs™ was confirmed with the bacterial contamination study. Thus, after collection, nylon flocked swabs used at a crime scene can be immediately placed in the plastic sample collection tube. This forgoes the need to dry the swab and also provides protection for the sample (Image 3).

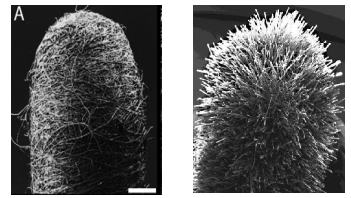


Image 4: SEM images of cotton swab (left) and nylon swab (right).

ACKNOWLEDGMENTS

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REFERENCES

1. Copan Flock Technologies website. Accessed 1 Aug 2013. http://www.copanflocktech.com/index.php/prod/sampleprep/nucleic/