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 Flock Technologies, Brescia, Italy), is made of parallel short nylon strands that are flocked onto a plastic stick, which lacks an inner core (Image 1). This study was to compare DNA recovery of cotton and nylon flocked swabs from lymphocytes collected from various substrates, which have been shown to affect DNA yield, and to determine the best extraction method for 4N6FLOQSwabs™. 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).

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™.

### 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 before spotting 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. The modified protocol was successfully developed.

#### Results

<table>
<thead>
<tr>
<th>Condition</th>
<th>DNA Recovery (%)</th>
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</thead>
<tbody>
<tr>
<td>Blood</td>
<td>60 ± 5</td>
</tr>
<tr>
<td>Nylon</td>
<td>75 ± 5</td>
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</tbody>
</table>

In our hands, across all the tested conditions, best DNA recoveries and highest DNA recovery from samples spotted directly in the tube (both blood and buccal) 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.

### Discussion

In our hands, nylon flocked swabs provided better DNA recovery from bloodstains. The increased 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 DNA yields 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 inconsistent results 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 nuclearic acid 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 (blood and buccal) 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 and highest DNA recovery from samples spotted directly in 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 stored in the plastic sample tube, also providing a protected environment for the sample.

### References


Acknowledgments: we would like to thank Copan Italia and Life Technologies for their support and advice on this project.