Determining the Ideal Swab Type for Collection of the Microbiome for Forensic Identification Purposes

Natalie M. Wise, Travis J. Worst, Jon E. Sprague, Crystal M. Oechsle
116 Life Sciences Building, Bowling Green State University, Bowling Green, OH 43403

OHIO ATTORNEY GENERAL'S CENTER for the FUTURE of FORENSIC SCIENCE AT BOWLING GREEN STATE UNIVERSITY

Introduction

In recent years, forensic scientists have begun examining novel methods, one of which is the use of the microbiome. The microbiome is made up of all the microorganisms living on or in the human body.[1] Research has already shown that it may be possible to use the microbiome as a unique identifier [2,3], to link cohabiting individuals [4], or even to connect a person with a location [5].

However, because use of the microbiome as a forensic tool is relatively new, some of the most basic research has yet to be completed. For instance, it has yet to be answered what the best collection tool might be. Therefore, this research focuses on determining the optimal swab type for collection and further analysis of the microbiome.

Here, a bacterium, *Proteus mirabilis*, was deposited onto each of four different types of swab: Puritan™ 6" Standard Cotton Swab w/Wooden Handle, Plasdent™ Maxapplicator™ 'Regular Size' (2.0 mm) Dental Applicators, Copan FLOQSwabs™, and Luna Innovations Incorporated™ Dissolvable Swabs. Cotton swabs were included due to their availability and widespread use in the forensic community, despite being known to not release or elute samples efficiently.[6] Dental applicators were included due to their small surface area, which may prevent samples from becoming entangled or trapped within the swab.[7] Generally, flocked swabs are designed with perpendicular fibers and no internal mattress core, this theoretically allows them to effectively collect and elute a sample.[8] The Luna™ dissolvable swab heads are made from cellulose acetate, a compound that is insoluble in common liquids, such as water and ethanol, but is soluble in buffers that contain chaotropic salts.[9] Chaotropic salts, like guanidinium thiocyanate are commonly used in commercially available DNA extraction kits.

Methods

After deposition of a predetermined amount of *Proteus mirabilis* stock onto each swab, the bacterial DNA was then extracted using the Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit, which is known to contain guanidinium thiocyanate.

Each round of extraction included one cotton swab, one dental applicator, one Luna dissolvable swab, one flocked swab, one positive control that was comprised of *Proteus mirabilis* stock in a tube without a swab, and a negative control. Seven replicate extractions were processed using the following method: Samples were incubated at 56°C in a mixture of 'Enhancer Solution' and Proteinase K before addition of 'Lysis/Binding Solution'. Samples were centrifuged using spin baskets. 'Magnetic Binding Beads' and 100% isopropanol were added. The DNA-bound beads were then washed repeatedly before the purified DNA was finally eluted.

Extracts were quantified using iTaq Universal SYBR Green Supermix (Bio-Rad) and Integrated DNA Technologies (IDT) 16S rRNA forward and reverse ReadyMade™ Primers on an Applied Biosystems QuantStudio™ 5 (QS5) instrument. The total DNA (ng) in each extract was calculated. Statistical analysis was performed using R freeware (v4.0.2). Between-group comparisons were conducted by ANOVA followed by a Tukey's Honest Significant Difference (HSD) *post hoc* test. Significance was set *a priori* at $p < 0.05$.

Results

FIGURE 1: SWAB TYPES EVALUATED

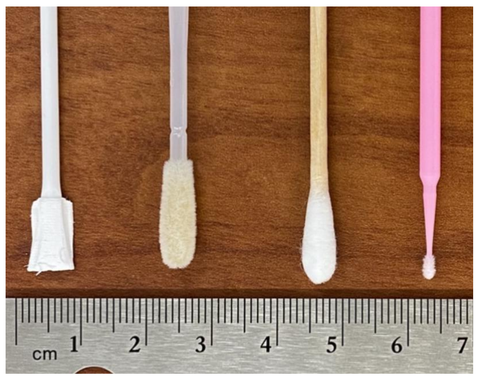

Figure 1. Pictured above are the four swab types evaluated in this study. From left to right: dissolvable, flocked, cotton, dental.

FIGURE 2: TOTAL BACTERIAL DNA YIELD FROM EACH SWAB TYPE

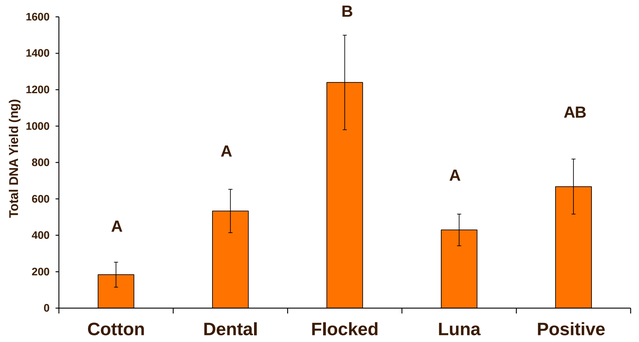

Figure 2. The above graph shows the total bacterial DNA yield from each swab type tested. Results displayed represent the average of the seven trials +/- Standard Error of the Mean (SEM). Letter designations represent Tukey's Honest Significance Difference (HSD) comparisons: the same letter designation means results are not statistically different; when letter designations differ between groups, the p-value is less than 0.05.

FIGURE 3: MELT CURVE

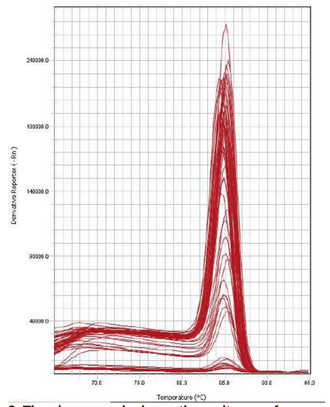

Figure 3. The above graph shows the melt curve from each sample. All samples fell within the expected temperature range of 84.4-85.3°C for *Proteus mirabilis* DNA[11]

Discussion

At first glance, these results may seem counterintuitive, as one might expect the highest yield to arise from the dissolvable swabs given that there should be no swab material remaining post-extraction. However, this might be partially explained by the fact that the dissolvable swabs we were working with were a prototype, which required manual shaving to ~⅓ the original size prior to use. Furthermore, all extraction volumes were doubled for the dissolvable swabs, and corresponding negative controls, so as not to saturate the system. It may be possible that these precautions did not sufficiently accommodate the dissolvable swabs, and the dissolved or partially dissolved material interfered with the extraction process. Additionally, as the MagMAX™ DNA Multi-Sample Ultra 2.0 Kit, which is designed for use with bacterial samples, is not routinely utilized in forensic analysis, the Luna™ dissolvable swabs have not been fully optimized for use with that kit. All of these factors could have contributed to the variability observed in the dissolvable swab group and the lower than expected yields.

On the opposite end of the spectrum, the cotton swabs performed roughly as expected, apparently trapping much of the DNA within the swab.[6,10] Due to the limited surface area of the dental swabs, one may have expected them efficiently release the biological material, yet they fared no better than the dissolvable swab and performed worse than the flocked swabs. This becomes interesting when one notes that similar recoveries were observed with all of the low/no surface area groups (dissolvable swabs, dental applicators, and the positive control) and that each of those sample groups were outperformed by the flocked swabs. We know that flocked swabs are made with nylon fibers that are positioned to keep the sample near the surface and readily available for elution. Perhaps, that is only half the story when it comes to the microbiome. We hypothesize that sample drying on the swabs contributes to lysis of the bacterial cells, which are protected by a peptidoglycan cell wall as well as two membranes in gram-negative bacteria. Additionally, cells may be more dispersed over the swab surface, which may have allowed better access to chemicals during the extraction process. It is also possible that the high concentration of DNA in the positive control saturated the surface of the magnetic beads during extraction process or that some DNA binding with the interior tube plastic resulted in sample loss.

Conclusions

There was no statistically significant difference in the total bacterial DNA yield between dissolvable swabs, dental applicators, cotton swabs, and the positive control. There was also no statistically significant difference in the total bacterial DNA yield between the positive control and flocked swabs. However, there was a statistically significant difference in the total bacterial DNA yield found between the flocked swabs and each of the other swab types: dissolvable swabs, dental applicators, and the cotton swabs ($p < 0.05$).

1. **The highest recovery, in total ng of *Proteus mirabilis* DNA, was obtained from Copan FLOQswabs™.**
2. **Flocked swabs yielded greater than 2-fold more *Proteus mirabilis* DNA than the next best performing swab type, Plasdent™ Maxapplicator™ 'Regular Size' (2.0 mm) Dental Applicators.**
3. **The *Proteus mirabilis* DNA yields from the *Puritan™ Cotton Swab, Plasdent™ Maxapplicator™ Dental Applicators, and Luna™ Dissolvable Swabs were not* significantly different from one another.**

Acknowledgements

This research was supported in part by The Katzner Graduate Student Research and Professional Development Award Fund (BGSU). Additional support from the Ohio Attorney General's Center for the Future of Forensic Science. Thank you to Luna Innovations Incorporated™ (Roanoke, VA, US) for providing the dissolvable swabs for this research.

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