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TECHNICAL NOTE

CRIMINALISTICS

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A Comparison of DNA Collection and Retrieval from Two Swab Types (Cotton and Nylon Flocked Swab) when Processed Using Three QIAGEN Extraction Methods

ABSTRACT: The Metropolitan Police Service currently uses cotton swabs to retrieve DNA for forensic profiling. Recently, a new nylon flocked swab type has become available from Copan (MicroRheologics, Brescia, Italy) that it is claimed, offers increased sample recovery and release yields. If true, the flocked swab may have important applications in DNA evidence retrieval. This study examines the DNA retrieval capability of cotton and nylon flocked swabs when extracted using three common extraction platforms (QIAcube, BioRobot EZ1 and manually processed QIAamp DNA investigator kit). Results indicate that both swab types are capable of recovering high percentages of DNA (>50%); however, the extraction platform selected was shown to have a significant effect upon DNA retrieval. Across all experiments, the cotton swab combined with the spin-column extractions was shown to be most effective, with the nylon swab and BioRobot EZ1 combination being the least effective. These findings illustrate the importance of extraction method selection.

KEYWORDS: forensic science, DNA extraction, nylon swab, flocked swab, cotton swab, QIAcube, BioRobot EZ1, DNA extraction, QIAamp DNA investigator kit

Swabs have been used for many years for DNA evidence retrieval by scene examiners and forensic scientists, enabling collection of a wide range of evidence types. Currently, the Metropolitan Police Service (MPS) uses sterile cotton swabs at crime scenes to collect DNA evidence. Cotton swabs have a mattress design, which refers to the way the cotton is tightly wound around the wooden/plastic shaft to make the bud (Fig. 1). It has recently been suggested, however, that swabs based on this design (i.e., those currently made from cotton or rayon) may not be particularly effective at retrieving (1) and later releasing cellular material (2) (even after vortexing [3]). In response, an alternative has been developed that utilizes a flocked swab design made from nylon (Fig. 1). The manufacturer (Copan [MicroRheologics, Brescia, Italy]) claims that the nylon flocked design enables rapid absorption by capillary action, and minimizes entrapment of collected samples by holding the sample close to the swab surface. As a consequence of the increased absorption, the manufacturer claims that only a single wet swab is required when sampling, instead of the wet and dry swab taken when using cotton swabs in many laboratories or at crime scenes. If true, this feature would reduce the time and cost associated for some police agencies and some laboratories.

While cotton swabs have traditionally been used for DNA retrieval at crime scenes, little is known regarding the suitability of the new nylon flocked swab as a tool for the collection of evidence at crime scenes. Previous research in the field of medicine has suggested that the nylon flocked swab is capable of collecting and releasing significantly more epithelial cells than rayon swabs (similar to cotton swabs in design) when nasopharyngeal and nasal swabs were taken from 16 volunteers (2). Similarly in a microbiological study of contamination recovery during environmental monitoring procedures, the nylon flocked swab was shown to collect and release 20–60% more micro-organisms from surfaces than other swabs types tested (1). The same study also concluded that the nylon flocked swab demonstrated "an instant and nearly complete release of absorbed samples of more than 80%" (1, p. 191). If similar results can be found when collecting and extracting forensic DNA samples, then there is certainly the potential that nylon flocked swabs could become a useful tool in DNA evidence retrieval.

To assess the suitability of the nylon flocked swab for the collection of DNA, two experimental setups were designed and run. Samples of high and decreased DNA concentration were used to replicate saliva stains often encountered. DNA samples were swabbed with either cotton or nylon swabs, and the DNA recovery rates compared. The two swab types were also assessed on their suitability for crime scene sampling, by considering ease of use, laboratory processing, and cost to purchase.

In addition, three QIAGEN (Hilden, Germany) DNA extraction methods were compared to assess efficiency (QIAamp DNA investigator kit [hereon designated "manual" extraction], BioRobot EZ1, and the QIAcube).

Obtaining usable quantities of DNA from forensic samples is important for downstream applications, and as a result, consideration needs to be given not only to the swab type used during sample collection, but also to the extraction method employed to extract and clean the DNA. Indeed, it is the combination of these

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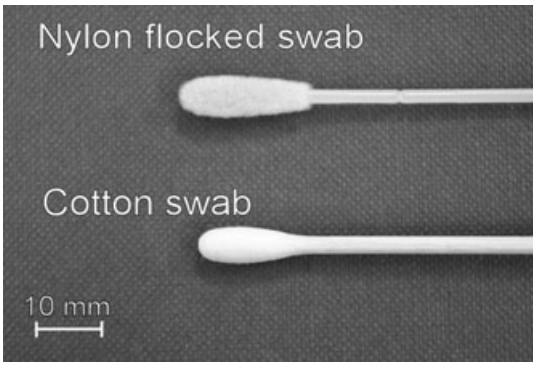

FIG. 1—*Cotton and nylon flocked swab designs.*

two factors, which will ultimately determine the quantity and quality of the DNA retrieved.

Given the recent reduction in cost, coupled with the relative ease of DNA extraction and genetic profiling, high-throughput laboratories are becoming increasingly common and as such scientists are turning to automated solutions to manage increasing forensic workloads (4–6). To this end, QIAGEN has introduced the BioRobot EZ1, and more recently the QIAcube, for use in automated DNA extractions (7). While the latter provides an automated solution for QIAGEN spin-column extraction (traditionally extracted manually), the BioRobot EZ1 is designed to rapidly process samples using single use reagent cartridges and protocols loaded onto a preprogrammed card. In both cases, DNA extraction relies on chaotrophic agents such as guanidine hydrochloride, which promotes lysis of cells, denatures proteins, and inhibits nucleases (5). While the manual and QIAcube extractions both use spin-columns with silica technology to isolate and purify the DNA, the BioRobot EZ1 uses paramagnetic bead technology to achieve the same goal (6). As a result, there is the potential for differences in the way these extraction methods handle the different swab types tested. This study sought to determine which swab type/extraction method combination yielded the best results, when collecting and extracting DNA from dry saliva stains.

Materials and Methods

High-Quantity DNA Samples

To investigate the effect of swab type and extraction method on DNA retrieval rates from samples with high quantities of DNA, a balanced experiment was designed. A saliva sample (2.5 mL) was collected from a single volunteer at a single time, diluted (1:1) with sterile distilled water, and vortexed thoroughly for 60 sec to ensure a homogenous solution. Fifty microliters of diluted saliva was then extracted using a QIAGEN QIAamp DNA investigator kit, processed using the "Isolation of total DNA from small volumes of blood or saliva" protocol, and quantified using an Applied Biosystems 7500 real-time polymerase chain reaction (PCR) system with Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Warrington, U.K.). Results of this quantification indicated that the volunteer saliva sample contained *c.* 20 ng/μL of DNA before being diluted (10 ng/μL after dilution). To create the high-quantity DNA samples (500 ng), it was decided that 50 μL of the diluted saliva sample (10 ng/μL) would be used. Consequently, 135 sterile 90 mm petri dishes were individually numbered, and 50 μL of diluted saliva was pipetted as a drop into the center of each petri dish and allowed to dry overnight. Care was taken to guarantee that the saliva stock sample was well mixed before each 50 μL sample was taken. A further nine petri dishes were also set up as control samples, which did not contain saliva samples.

Decreased Quantity DNA Samples

To emulate samples with decreased quantities of DNA, a second experiment was designed and run, in which saliva from a second volunteer was collected, and DNA was extracted and quantified using the same methods as described previously. The sample was then diluted to a DNA concentration of 1.5 ng/μL. *c.* 50 ng of DNA was applied to 60 sterile 90 mm petri dishes. Six control plates were included in the decreased quantity DNA experiment, which did not contain saliva samples.

Sample Collection

In both experimental setups, samples were left overnight in a lamina flow hood, after which dishes were checked to ensure that saliva samples had dried. Each petri dish was then swabbed using a single, predesignated swab type. To maximize the chances of DNA retrieval from each petri dish, and to ensure that all swabs were treated equally, a standardized swabbing technique (similar to that used at crime scenes) was employed. For this, each swab was removed from its packaging and two drops of sterile distilled water were added to the swab bud to provide enough moisture for the subsequent swabbing procedure. It should be noted that while wetting the swabs was a simple task with the cotton swab (because of its absorbent nature), the nylon flocked swab design required far more time and care to ensure that the swab absorbed the specified amount of sterile distilled water.

Once wet, a single swab was then used to collect the saliva from a specific single petri dish. To ensure a fair and equal swabbing process, each dish was swabbed with 15 strokes in a single direction, the petri dish was then rotated through 90° and a further 15 swabbing strokes were made. All the time the swab was rotated to ensure an even distribution of the sample onto the bud. Firm swabbing of the dish surface was made difficult using the nylon flocked swab because of the flexible nature of the plastic shaft. By comparison, the wooden shaft of the cotton swab type allowed simpler swabbing.

When completed, swabs were placed back inside their packaging (or provided 2 mL Eppendorf tube in the case of the nylon flocked swabs) and stored at 4°C. Swabs taken from control plates were treated exactly the same as those used for the sample dishes.

Sample Extraction

In both experimental setups, collected swabs were extracted the same day to minimize any effects because of degradation. Prior to extraction, cotton swab heads were removed from swab shafts and placed into sterile 2 mL Eppendorf tubes. This involved shaving the cotton from the wooden shaft with a sterile scalpel blade. In contrast, the nylon flocked swabs required no further attention as they were already contained within a 2 mL Eppendorf tube ready for the extraction. It should be noted that nylon flocked swabs were simple to detach from the swab shaft as they have been designed with a specific break point directly above the flocked bud.

Manual and QIAcube Automated Extraction

For the extraction of DNA from swabs (both high and decreased DNA quantities), it was decided that both the manual extraction

and the QIAcube automated extraction should be run using the same QIAamp DNA investigator extraction kit and protocol to allow direct comparison to be made between these extraction methods. In each case, 580 μL of buffer ATL (QIAGEN) and 20 μL Proteinase K (20 mg/mL) were added to each sample. Samples were then vortexed thoroughly for 15 sec and left to incubate on a thermo-mixer (14,000 × *g*) at 56°C for 1 h. After this time, QIAcube samples were loaded onto the platform and run using the "Surface & buccal swabs" protocol, while samples for manual extraction were processed according to the manufacturer's instructions for "Isolation of total DNA from surface and buccal swabs" protocol.

BioRobot EZ1 Extraction

To each of the samples for BioRobot EZ1 extraction, 290 μL G2 buffer (QIAGEN) and 20 μL Proteinase K (20 mg/mL) were added, along with 290 μL of sterile distilled water. These samples were then vortexed for 15 sec and placed onto the thermo-mixer according to the protocol instructions (56°C for 15 min, followed by 5 min incubation at 95°C). After this time, all samples were briefly spun down and loaded onto the BioRobot EZ1 for extraction using the EZ1 DNA investigator kit and the DNA purification ("Tip Dance") protocol.

The elution value was set as 50 μL of water for each of the extraction methods, and once extracted, all samples were stored at −20°C until quantification was carried out.

DNA Quantification and Analysis

DNA was quantified using an Applied Biosystems 7500 real-time PCR system with Quantifiler® Human DNA Quantification Kit. Concentrations of DNA (ng/μl) were recorded, and statistical analyses were conducted using MINITAB v.15 (MINITAB Ltd., Coventry, U.K.). Given the factorial nature of the experimental designs, each data set was analyzed in turn using a univariate two-way balanced analysis of variance (ANOVA), with swab type and extraction method as factors. In each case, the interaction between these factors was also calculated to determine whether DNA recovery from swabs depended on the extraction method used or whether similar results were observed regardless of the extraction method.

To determine statistical differences in DNA yield between swab types within extraction methods, a within treatment one-way ANOVA was performed for each of the extraction methods, with swab type as the factor. A *p*-value of <0.05 was taken as statistically significant in all analyses.

DNA Amplification and Profiling

A proportion (10%) of the samples were amplified using AmpF*ℓ*STR®SGM Plus® kit (Applied Biosystems) according to the manufacturer's instructions (total reaction volume = 25 μL). Amplified products were profiled using an ABI 3130*xl* genetic analyzer (Applied Biosystems) and results were analyzed using GENEMAPPER ID v.3.2 (Applied Biosystems).

Results

High-Quantity DNA Samples

Analysis using the two-way ANOVA for samples containing a high quantity of DNA revealed a significant interaction effect between swab type and extraction method ($F_{2,71} = 25.52$, $p < 0.001$), indicating that different combinations of swab type/extraction method yielded different results. Figure 2 shows the nature of this interaction by comparing each combination type. To determine the percentage recovery achieved by each combination of swab type and extraction method, data were standardized by converting DNA values to a percentage of the starting stock DNA used.

Results show that the combinations of cotton/QIAcube and nylon/manual yielded the highest quantities of DNA with percentage recovery values of 64.5 ± 8.0% and 59.3 ± 10.0%, respectively. By comparison, swabs extracted using the BioRobot EZ1 resulted in the lowest recovery of DNA, with the nylon/EZ1 proving to be the least effective combination (15.9 ± 4.4%).

In addition to comparing each swab type/extraction method combination, this study also conducted analysis to assess swab type performance within each extraction method. Comparison using one-way ANOVA revealed that the nylon flocked swab produced significantly higher yields of DNA than cotton swabs when both were extracted using the manual extraction method ($F_{1,23} = 26.25$, $p < 0.001$). In contrast, the cotton swab was shown to perform significantly better than the nylon flocked swab when both were extracted using the automated platforms (QIAcube [$F_{1,23} = 12.46$, $p < 0.001$] and the BioRobot EZ1 [$F_{1,23} = 16.17$, $p < 0.001$]). Extracted control samples showed no quantifiable levels of DNA when examined using Quantifiler technology.

Decreased Quantity DNA Samples

Results of the two-way ANOVA for decreased quantity DNA samples, like the high DNA quantity samples, indicated that different combinations of swab type/extraction method yielded different results, as shown by the significant interaction effect between swab type and extraction method ($F_{2,59} = 4.9$, $p < 0.05$). The combination that produced the highest percentage DNA recovery was the cotton/QIAcube (66.4 ± 10.9%), while the least effective

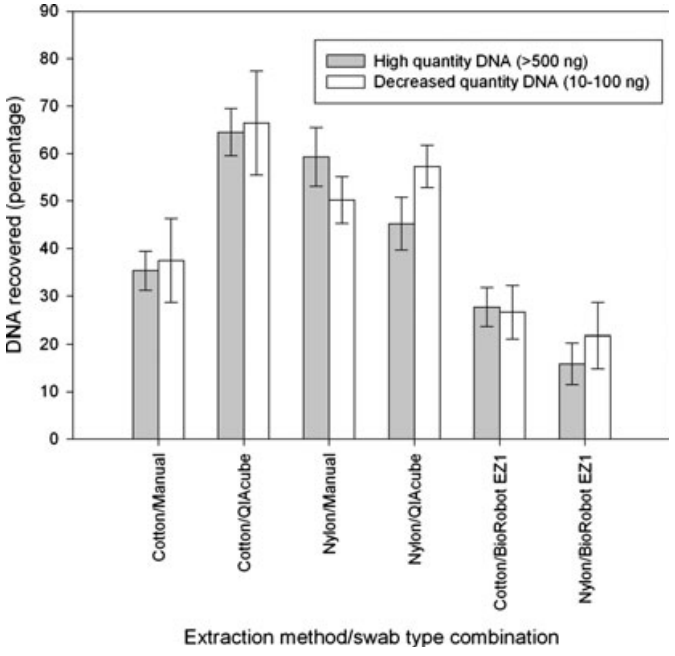

FIG. 2—*A comparative bar chart showing the proportion of high and decreased quantities of DNA recovered (compared to stock) by each swab type/extraction method combination. Error bars represent 95% confidence intervals.*

combination was shown to be the nylon flocked swab type with the BioRobot EZ1 (Fig. 2).

Results from the comparison of swab types within the extraction methods revealed that when using the manual extraction method, the nylon flocked swab performed significantly better than the cotton swab type ($F_{1,19} = 6.1$, $p < 0.05$). In contrast, no significant difference was observed between swab types when extracted using either the QIAcube ($F_{1,19} = 2.3$, $p = 0.147$) or the BioRobot EZ1 ($F_{1,19} = 1.2$, $p = 0.291$), although in both cases, the cotton swab was shown to perform marginally better than the nylon flocked swab type. Extracted control samples showed no quantifiable levels of DNA when examined using the Quantifiler® Human DNA Quantification Kit.

DNA Profiling

All samples amplified using the AmpFℓSTR®SGM Plus® system produced the expected DNA profiles. There was no evidence of contamination or degradation in any of the samples.

Discussion

Experiments conducted during this study were designed to compare the DNA retrieval ability of two different swab types (cotton and nylon flocked) when extracted using one of three QIAGEN extraction methods.

Previous research has suggested that the cotton swab type may not be the most effective design for retrieving and releasing samples (2). By comparison, the recently developed nylon swab with its flocked design is claimed by the manufacturer to offer "rapid absorption of samples" and "superior sample release." Indeed, several studies in the field of medicine have shown the nylon flocked design to perform significantly better than other swab types tested (2,8). As a consequence, one may have expected the nylon flocked swab to outperform the cotton swab, when applied to forensic applications. However, DNA retrieval results obtained during this current study indicate that both the cotton and the nylon flocked swab types are capable of retrieving and releasing a high percentage of the DNA from dry, saliva stains, containing both high and decreased amounts of DNA. One explanation could be the way in which the different swab types are processed. In many forensic laboratories, it is common practice to "shave" the cotton from the wooden shaft prior to lysis. This method will no doubt help increase DNA recovery, as removing the cotton from the shaft in this way would likely promote better accessibility of the cells to lysis. However, in some laboratories, practices require users to cut off the swab heads with the wooden or plastic shaft still attached. In such cases, the spool remains undisturbed and DNA may become entrapped in the cotton fiber network, resulting in reduced DNA yields. With this in mind, laboratories should consider an alteration in the way cotton swabs are processed prior to lysis to ensure that maximum yields of DNA are obtained. Similarly, it is recommended that laboratories attempt to optimize extraction protocols for the nylon swabs that are not based on the manufacturers' instructions. With further investigation, lysis conditions could be found that may promote increased yields than those investigated during the course of this study.

Another possible explanation for these contradictory results is that much of the previous research has used the nylon flocked swab to sample an abundant quantity of moist test material (2,9,10). By comparison, samples collected at crime scenes will tend to be in a dried state and in small quantities. Thus, the collection of these samples often requires the addition of moisture and a firm swabbing action to remove the sample. Even though this study has shown the nylon flocked swab to be as effective as the cotton swab in many instances, the complications observed during this study relating to the application of moisture to the nylon bud, plus the difficulty noted during the physical act of swabbing (because of the flexible nature of the plastic shaft), mean that the current design of the nylon flocked swab is not particularly suited for the collection of dry forensic samples at crime scenes. While these issues are easily overcome in a laboratory environment, the need for ease of use and a simple swabbing process in the field currently counts against the nylon swab as a tool for crime scene use. With this in mind, it is recommended that a redesign of the swab shaft and a standard protocol for applying moisture to the swab should be considered in the future to make the nylon swab a viable option for crime scene use. Furthermore, these steps may also help to increase recovery yields which will be of great benefit when retrieving decreased quantity DNA samples commonly encountered during crime scene investigations.

Another important factor to consider when assessing swab suitability for forensic applications is the cost. A comparison between the two swab types has shown that the newly designed nylon flocked swab is over six times more expensive than the currently used cotton swab. Given that the MPS submits more than 6000 volume crime samples in the form of swabs every year (statistics from 2008), this difference in cost is a significant consideration when assessing the suitability of the nylon flocked swabs for wide-scale forensic applications. This increase in cost will be counteracted to some extent by the fact that only a single wet nylon swab is required when swabbing, instead of the standard wet and dry which is required when using cotton swabs. However, this will only stand true for police agencies and forensic laboratories that have adopted the two-swab technique, which many around the globe have not.

Another consideration is that the nylon swabs appear to be quick and easy to process in the laboratory, as the flock can be easily removed owing to the break point in the shaft. With this in mind, the current design of the nylon swab may be more suitable for evidence retrieval in the laboratory than it would be at crime scenes.

Perhaps the most interesting finding highlighted during this study is that DNA recovery appears to be influenced by the combination of extraction method and swab type used. For example, when extracting high-quantity DNA samples using the QIAGEN automated platforms (QIAcube and BioRobot EZ1), the cotton swab was shown to perform significantly better than the nylon flocked swab. However, the nylon flocked swab was shown to retrieve significantly more DNA than the cotton swab when both swab types were processed using the manual extraction procedure. It would therefore appear that to accurately assess DNA retrieval from swab types, one must also consider the DNA extraction method used.

Automated extraction techniques (like the QIAcube and BioRobot EZ1 tested here) allow high-throughput laboratories to devote less man-hours to the extraction of samples. To be effective in forensic laboratories, however, automated extraction systems need to fulfill certain criteria. In a study by Anslinger et al. (4), it was suggested that automated systems should be capable of extracting DNA to an equivalent quality and quantity to that of manual extraction methods. While results from this current study have shown the automated QIAcube to be comparable to the manual extraction method, it is interesting to see that the automated BioRobot EZ1 extracted significantly less DNA on average than either the manual extraction or the automated QIAcube, regardless of the quantity of DNA used in the sample preparation, or the swab type used. One possible explanation for this reduction in yield could be

the relatively short incubation time (outlined in the EZ1 Investigator protocol) for the lysis step (15 min at 56°C, then a 5 min step at 95°C). By comparison, the protocol for the manual and QIAcube extraction instructed that samples should be left to incubate for at least 1 h at 56°C. This difference in incubation time could certainly result in reduced yields of DNA. Therefore, a study of incubation time was conducted (results not shown) and it was found that increasing the incubation time to an hour had no significant effect upon the quantities of DNA extracted using the BioRobot EZ1 ($F_{1,11} = 1.97$, $p = 0.191$). Therefore, reduced incubation period cannot be the explanation for the decreased extractions yields observed when processing samples on the BioRobot EZ1. This is not the first time the efficiency of the BioRobot EZ1 has been called into question. A previous study found the EZ1 platform to consistently recover less DNA than traditional organic extraction methods tested (10). In this instance, it was found that further optimization (via the addition of carrier RNA to the lysate) was required to increase yields. However, given that the addition of carrier RNA was also shown to increase DNA yields in all other extraction methods tested, the BioRobot EZ1 was still concluded to underperform when compared to other extraction methods. Furthermore, care should be taken when adding RNA to increase sample yields, as its introduction has been shown to cause problems in downstream applications, particularly PCRs, where it has been closely linked with causing inhibition (11).

While the precise explanation for reduced yields from the BioRobot EZ1 is not clear at this time, it is recommended that further investigation be undertaken to assess the BioRobot EZ1 for forensic applications by examining its ability to extract DNA from a wide range of different forensic samples. These results should then be compared to yields obtained using alternative extraction methodologies.

Conclusion

Based on the results presented here, there is not enough evidence to suggest that the nylon flocked swab should be considered for use at volume crime scenes as a replacement for the currently used cotton swab type. However, there is some evidence to suggest that the nylon flocked design may serve as a useful tool in a controlled laboratory environment. For this potential to be fully realized, however, it is recommended that the swab shaft undergo a redesign, laboratories develop optimized protocols, and additional investigation be conducted into the nylon swab and its retrieval efficiency across a range of different sample types and concentrations. Furthermore, if nylon swabs are to be processed in the laboratory, care must be given when selecting the extraction method, as this study has shown significant differences between DNA retrieval when processed using three different QIAGEN extraction methods.

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