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A holistic approach for the selection of forensic DNA swabs

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ABSTRACT

In the present study, we compared the performance of five different ISO 18385 certified forensic swabs for DNA sampling in practice over a time period of five months. Comparisons were made for DNA profiling success rates, measured as the percentage of CODIS (Combined DNA Index System) suitable profiles as well as for practical suitability during sampling at the scene, measured through a survey among collaborators. More than forty members of our crime scene investigation (CSI) unit took part in the test series and provided structured feedback concerning different aspects of swab handling. A total number of 1094 "touch" DNA samples have been subjected to DNA analysis. Swabs performed significantly different in terms of DNA profiling success rates. We also observed significant differences in DNA extraction efficiency between swabs. The evaluation by the collaborators of various aspects of handling differed significantly between swabs. We can assume that a more convenient handling decreases the risk of contamination or sample mislabelling and increases sampling efficiency and staff satisfaction. Our results demonstrate that the selection of disposable sampling devices such as forensic swabs for DNA sampling should be made based on a holistic approach. To be able to select the best performing swab for a given combination of CSI and DNA laboratory procedures, it might not be sufficient to only perform DNA extraction comparisons and trace sampling under controlled laboratory conditions.

1. Introduction

There are currently many different swabs for forensic DNA sampling on the market. In Switzerland, several police departments switched in recent years from cotton to nylon-based swabs, what inspired us to also consider a change of our current sampling setup.

Many studies have been published in the forensic field with respect to the choice of the best swab for DNA sampling. Many of these studies have been done based on rich, reproducible traces such as saliva, blood or other ([1–10]). Although this is probably the most common type of trace analysed in DNA labs today, fewer studies have focused on contact or "touch" DNA given the difficulty of reproducing contact traces with standardized amounts of DNA in the laboratory ([11–18]). A DNA trace sample is commonly referred to as contact or "touch" DNA, as we call it here in the following, if it is sampled upon the suspicion of a skin contact between a person of interest and an item presumably touched in the course of a crime. The nature of the DNA-bearing biological material left behind by such a contact is not entirely clear, but it is often assumed to be derived mainly from epithelial cells [19]. For the sampling of "touch" DNA, several of the study authors above gave recommendations for certain swab types, based on their results. Some authors recommend the use of nylon swabs ([3,5,7,11,14]) while others recommend cotton swabs ([12,16]). Finally, the authors of some studies show no preference for either type of swab ([1,6,10,15]).

Based on the various published studies, we must conclude that there is no consensus on the type of swab to use (cotton or nylon). Only few studies have investigated other types of swabs (foam, polyester, viscose, etc.) and their results are inconclusive ([2,7,9,12,13,15]). However, there is a consensus that a drying system for the swab is required, either passive (air-permeable packaging) or active (desiccant in the tube), to ensure the preservation of the collected DNA [10].

In summary, the study of Bonsu et al. [18] highlights that the choice of swab depends on the type of surface to which it is applied. In particular, it is emphasized that cotton swabs are traditionally preferred for the collection of biological fluids, due to their large potential for the absorption of liquid. In addition, they are inexpensive and, given the large quantities used, more sustainable. One of the problems related to cotton swabs is that DNA bearing

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Table 1
Swabs selected for testing.

Swab	Type	AKA
ForensiX 9021040	Cotton	Swab 0
Bode SecurSwab 2	Cotton	Swab 1
Copan 4N6 FLOQSwabs®	Nylon	Swab 2
ForensiX 9022015	Cotton	Swab 3
Sarstedt 80.629	Viscose	Swab 4

material could be more efficiently retained by the cotton fibres and might therefore not be released during extraction. This is one of the main advantages advertised by the manufacturers of flocked nylon swabs, on which fibres are not packed but arranged more like on a brush, therefore more easily releasing sample material for DNA extraction [20].

However, an important aspect that has been largely neglected in most of the existing studies is the practicality of the various swabs for the officers using them in the field, i.e., crime scene investigators. The purpose of this paper is to provide some guidance to CSI teams interested in changing their swabs based not only on DNA sampling and extraction efficiency but also on aspects of user comfort. We therefore assessed the performance of five different swabs in real criminal casework over a period of five months.

2. Material and methods

2.1. Tested swabs

Table 1 shows the swabs selected for the test series. The swab currently used by our department, ForensiX 9021040 (Swab 0), was used as reference standard. Fig. 1 shows a picture of all five swabs we tested.

2.2. DNA extraction and analysis

Swab heads were cut and extracted with the AutoMateExpress™ device and the PrepFiler Express™ Kit (both Thermo Fisher, US), with an elution volume of 50 µl, representing our standard lab procedure for swabs from touched surfaces. Internal validation has demonstrated the efficiency of the method for cotton swabs [21]. DNA was quantified by Real-Time-PCR (qPCR) using the Quantifiler® HP Kit from Thermo Fisher on a 7500 RT PCR System (Thermo Fisher, US). DNA profiles were established by multiplex-PCR using the AmpFlSTR® NGM Select™ Kit (Thermo Fisher, US) in a total reaction volume of 25 µl (at least two independent amplifications). A maximum of 0.5 ng DNA was amplified per reaction, using the maximum sample volume of 10 µl for samples with DNA-concentrations below 50 pg/µl. In line with our standard operating procedures for casework, all samples with a DNA concentration below 20 pg/µl were amplified with 32 instead of 30 PCR cycles. Capillary electrophoresis was run either on a 3130xl or on a 3500xl genetic analyzer (Thermo Fisher, US). Signal interpretation was performed with Genemapper ID-X, v1.6 (Thermo Fisher, US). All peaks above 50 rfu (3130xl) and 100 rfu (3500xl) were considered as true alleles.

2.3. Test for extraction efficiency

Volumes of 10 µl from a single blood sample, diluted 1:10, 1:100 and 1:500 with saline solution were pipetted on the different swab heads, the swabs transferred to their tubes or cardboard boxes (swab 0) and dried for 7 days. We analysed four swabs per type and dilution step.

2.4. Field testing procedure

For the practical tests, 500 swabs of each type were provided in turn. They were used for routine sampling in real-life crime scene investigation until the stock was exhausted and then replaced by the next ones. This theoretically allowed each collaborator to test each swab at least once. In addition, the swabs were also deposited in the laboratories of the forensic section. This also allowed the handling of the swabs to be tested in these facilities. Since the new swabs were not supplied as part of a sampling kit (with the exception of the Copan), an internal kit was produced for testing. This kit consisted of a package with 20 C6-format envelopes and 20 double ID labels that can be stuck on the swab, 20 vials of water and 20 seals.

All casework was included in the swab rotation without preselection of particular case types. As provided by our usual sampling procedure, staff were free to choose their sampling method (single swab, double swab). In sake of a more efficient processing [22], the internally recommended method is to use only one swab, wet only one side and wipe the trace first with the wet, then with the dry side of the swab. However, depending on the size of the sampled surface area, double swab technique is frequently used as well for the cardboard box stored swabs (23% of the “touch” DNA traces). For swabs stored in plastic tubes, no double swab technique has been applied. Collaborators were advised to use nylon swabs with less water than cotton and viscose swabs.

At the end of the test-sampling period, a form was filled by each collaborator to obtain an evaluation of the tested product. Crime scene investigators were asked to rate the different criteria between 1 and 6, with 6 being the best grade, corresponding to the Swiss school grading system. The criteria to be evaluated were the following: transport to crime scene, handle stability, handle length, swab head quality, moistening, storage system, labelling, kit packaging (swab, envelope, label and water), waste, archiving. Alongside the grading, we asked the participants to give a weighting score from 1 to 10 for each criterion to determine its importance. The mean weighting score over all participants was then used to obtain the weighted average of the grades for each swab and criterion. This evaluation was characterised as an objectivized evaluation.

Collaborators also had to vote for the swab that they would choose themselves. This evaluation was characterised as a subjective evaluation.

The evaluation period was from February 4th to June 30th 2022. In total, 44 collaborators participated in the tests and 1296 traces were analysed by the Institute of Forensic Medicine, of which 1094 were "touch" DNA samples not sampled from human bodies and were included in the present study. The overall number of traces collected could not be documented as some swabs were used for demonstration purposes and others were returned or destroyed. However, virtually all 2000 available swabs of types 1–4 were used up by the CSI unit.

For the swabs sent to the Institute of Forensic Medicine for analysis, the success rate for the establishment of CODIS suitable DNA profiles has been used as a measure for the assessment of the swab performance. Since perpetrators are not known in most cases, the percentage of trace profiles entering the database directly determines the chances for a successful DNA based investigation. CODIS criteria in Switzerland are a minimum of 6 typed loci for single or major profiles and 8 loci for 2-person mixtures. For a locus of a single or major profile to pass internal quality guidelines, peak height ratio for heterozygotes must be at least 60% and the contributor ratio should be at least approximately 3:1.

2.5. Statistical analyses

To assess whether there was a significant effect of the swab type on the grade for a given survey item, we modelled the data using a

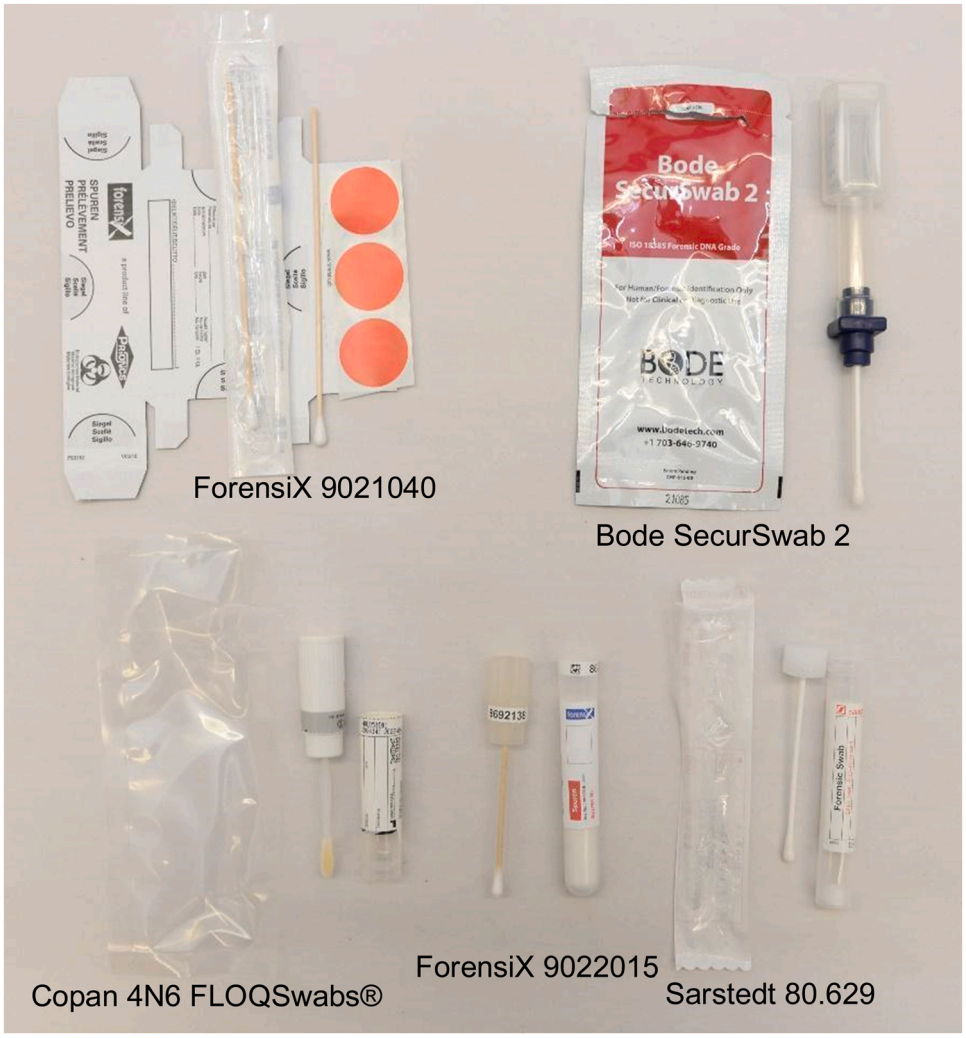

Fig. 1. Swabs selected for testing.

mixed-effects linear model per item. Let y_{ij} denote the i^{th} grade given by participant j, then the model equation is:

$$\begin{cases} y_{ij} = \beta_0 + \beta_1 S_{ij} + u_j + \varepsilon_{ij}, \\ u_j \sim \mathcal{N}(0, \sigma_u^2),\ \varepsilon_{ij} \sim \mathcal{N}(0, \sigma^2), \end{cases}$$

where β_0 is the intercept, β_1 the fixed effect associated to the swab type S_{ij}. u_j is a random effect on the intercept associated with the survey participant, and ε_{ij} the residual error, both are assumed to follow a normal distribution $\mathcal{N}$ of mean 0 and variance σ^2.

To assess the significance of the swab type effect, we performed a likelihood ratio test to test the difference between two nested models: the model described above and a null model excluding the fixed effect associated to swab type. The test statistic follows a chi-square distribution from which we computed p-values.

To better understand the differences between swab types on each survey item, we then performed post-hoc comparisons using Tukey's range test. The means of all swab type pairs were compared, and significance groups were built using the algorithm to build Compact Letter Display (CLD) proposed by Piepho [23].

T-Tests were performed in Microsoft® Excel®. All other statistical analyses and related graphical representations have been performed using R version 4.1.2 [24] and the following R librairies: *broom.mixed*, *dplyr*, *ggplot2*, *lme4*, *multcomp*, *readxl*, and *tidyr*.

3. Results

3.1. Swab selection

It is evident that the type of swab should be at least as safe and effective as the current method for DNA analysis. For the practical tests, we had to limit ourselves to a manageable number of different swabs, selected based on several criteria:

Certification for DNA-free production: The prevention of sample contamination is one of the major challenges, when dealing with trace amounts of human DNA. Therefore, only DNA-free certified (ISO 18385) swabs will be evaluated and sterile-only certification will not be accepted.

Swab head: As already mentioned in the introduction, it is not possible to determine from the literature which type of swab should be preferred. Therefore, we will test in parallel, cotton, viscose and nylon swabs, to observe the differences in use. Other types of swabs (foam, polyester, etc.) seem rather exotic to us and are not commonly used by our Swiss or European colleagues. In the interest of possible cooperation with external institutes, it is advisable to stick with well-known materials. Therefore, these other types of swabs were not considered in the practical tests.

Storage system: For reasons of saving time and reducing risk of contamination, the storage system for the swab should not need to be folded. It should provide the possibility to dry the swab (passive

or active system) and it should be easy to label without confusion. Too narrow tubes should be avoided, because it is difficult to insert the swab without touching the edges, which means a risk of contact and therefore increased risks of contamination and of loss of material when the swab is inserted or withdrawn.

Handle: The handle should be stiff enough to apply some pressure to the trace, but flexible enough not to break during sampling. A short handle also offers the possibility of more force and easier insertion into the tube, which should limit contamination. For these reasons, only swabs with a short handle were chosen for the practical part.

Guaranteed supply: To ensure that sufficient material is always available, swabs should come from a company with a solid reputation and assumed good financial standing. An asset is the existence of a well-established distribution network in Europe, if possible in Switzerland.

Compatibility with established extraction methods: Efficient sampling is useless if DNA extraction is not efficient downstream. The selected swabs were therefore checked for compatibility with the established extraction method.

The four swabs that fulfilled most of our criteria and that were selected for the comparison with our current swab (Swab 0) are listed in Table 1 and shown in Fig. 1.

3.2. Extraction efficiency

DNA extraction efficiency is not only dependent on the type of swab used, but especially on the combination of swab and extraction method [1]. We therefore had to check whether all the swabs are compatible with our established DNA extraction procedure. DNA extraction efficiency, tested on different blood dilutions, clearly demonstrates that the smallest amount of DNA can be retrieved consistently from Swab 0, the one currently in use (Fig. 2). Compared to Swab 0, significantly more DNA was extracted from Swabs 1, 3 and 4 from the 1:10 dilution samples, from all other Swabs from the 1:100 dilution samples, and for Swabs 1 and 3 from the 1:500 dilution samples. Swab 1 outperformed Swabs 2 and 3 for the 1:10 dilution and Swabs 2 and 4 for the 1:500 dilution (all p-values < 0.05). All other two-sided pairwise t-tests were not significant ($p > 0.05$).

3.3. DNA profiling success rates for casework samples

Since most of the traces secured and exploited (80–90% according to our internal data) concern contact traces and these represent a major challenge compared to other types of biological samples, only this type of trace was observed for the evaluation of the results (1163 out of 1296 traces). In addition, we can reasonably expect larger DNA amounts for contact traces sampled from the human body (e.g. in cases of assault). Therefore, those samples (69 out of 1163 “touch” DNA traces) were also not included in the analysis. The final number of swabs retained for this research were 1094, distributed as follows: Swab 0 = 519, Swab 1 = 149, Swab 2 = 197, Swab 3 = 136, Swab 4 = 93. The differences in success rates for the establishment of CODIS suitable DNA profiles from contact traces are displayed in Fig. 3. Profiles were considered CODIS suitable if they fulfilled the Swiss CODIS entry criteria, in combination with our lab internal quality criteria, as stated in the Material and Methods section.

All the new swabs appear to perform significantly better than Swab 0 (two-sided Welch’s t-test, $p < 0.001$ for Swabs 1–3 and $p < 0.05$ for Swab 4). No other pairwise comparisons between swabs were statistically significant. The gain in database suitable profiles of Swab 3 over Swab 0 is 102%. Swab 1 and Swab 2 follow with 74% and 71% increase, respectively. Swab 4 is in fourth place, but still 51% better than Swab 0.

3.4. Staff survey

The evaluation form was forwarded to 44 staff members. A total of 42 returned the form, reflecting a response rate of 95%. The results of the questionnaire are summarized in Fig. 4. Significant differences were observed for almost all criteria (Fig. 5).

The results of the collaborators’ votes (subjective evaluation), show that 2/3 would either choose swab 4 first (15 votes) or keep the current swab 0 (11 votes). Swabs 1–3 obtained one, four and eight votes, respectively. Three participants voted blank.

Looking at the results of the weighted averages (objectivized evaluation), the current swabs (Swab 0) are ahead with a slight advantage over Swab 4 and 3.

In all cases, the swabs 1 and 2 are in 5th and 4th place, respectively.

The importance of each criterion (weighted score) for collaborators is documented in Fig. 4. This shows that the five most important factors for the staff are: handle stability (8.72), swab head quality (8.63), labelling (8.27), archiving (7.65), and storage system (7.59).

3.5. Problems encountered and feedback from staff

Several observations were made during the course of the project, which are reported in the following:

For several staff, the envelope system proposed by this project to package the Swabs 1, 3 and 4, which allowed them to be labelled and sealed, was uncomfortable. Once the swab was placed inside, it was difficult to have a flat surface on which to write the notes. Also, the C6-sized envelopes took up a lot of space in the case files.

Swab 2 were supplied with a double label system for the swab number, so that part of the label could be peeled off and stuck on the envelope. This label was relatively difficult to peel off the swab, especially when wearing gloves.

For some staff, swabs with a short handle are inconvenient, especially to reach difficult areas.

There have been several occasions when Swabs 1 have come loose from their handles, rendering them unusable.

While writing this article, we were informed by Thermo Fisher, the parent company of the ForensiX and Copan swabs, that the production of the ForensiX product line will be discontinued from the end of 2023. This includes test swabs number 0 and 3. However, in a letter dated January 9, 2023, we received information from the company Voigtländer that the latter would take over the production of Swab 3 in its name from the manufacturer, guaranteeing the same product quality. The future name of the swabs is not yet known at the time of this publication.

4. Discussion

4.1. DNA extraction efficiency

We can conclude that the established PrepFiler Express™ extraction protocol is compatible with all tested swabs. However, the extraction efficiency is significantly increased for the four new swabs, compared to Swab 0 (Fig. 2). DNA extraction was most efficient from Swab 1. This was a bit surprising to us, since we would have expected the most efficient release of biological material from Swab 2, since this swab has been specifically designed to more readily release the biological material sampled with [20].

We are aware of the possibility that extraction efficiency for blood dilutions might not be exactly the same like for "touch" DNA traces. However, "touch" samples are very difficult to standardize for a meaningful comparison. We would expect larger discrepancies in extraction efficiency between swabs used for dry swabbing and swabs with pipetted blood dilutions, because with dry swabbing we

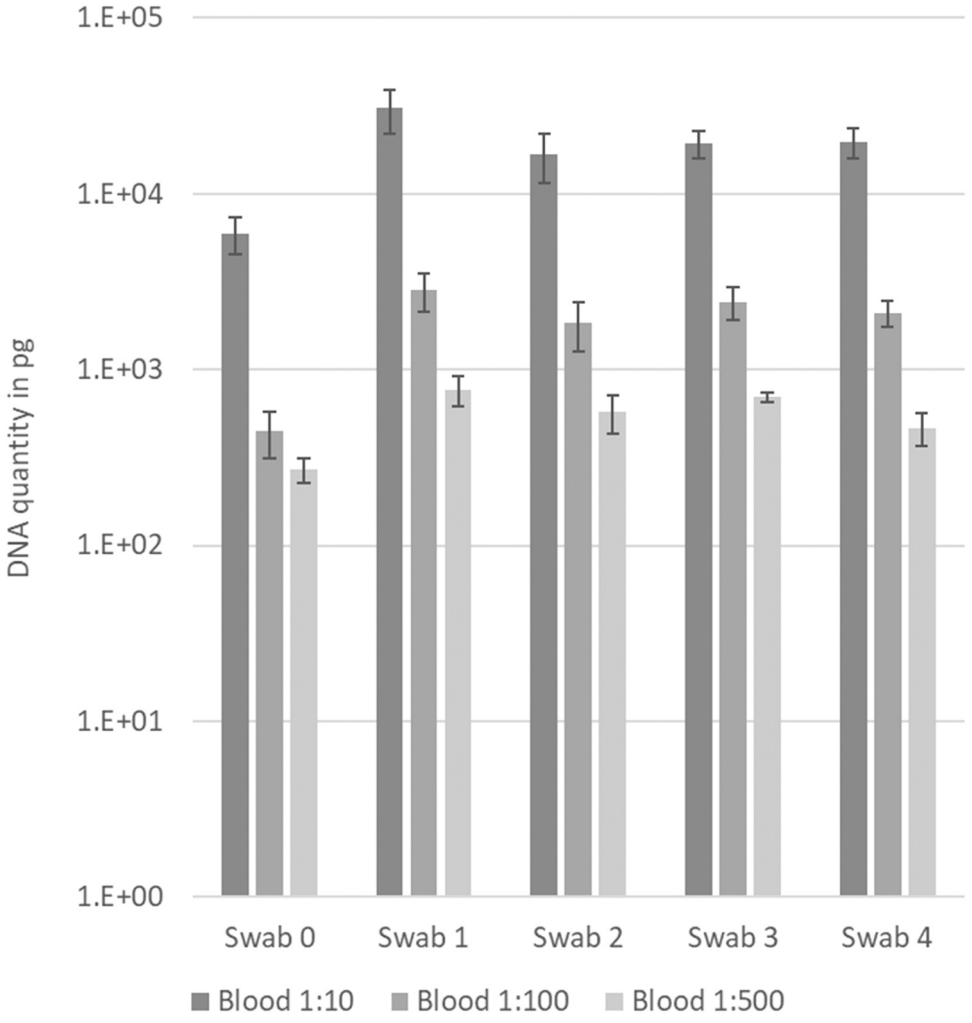

Fig. 2. DNA extraction from dilution series in pg DNA (log10 scale). Error bars represent standard deviations.

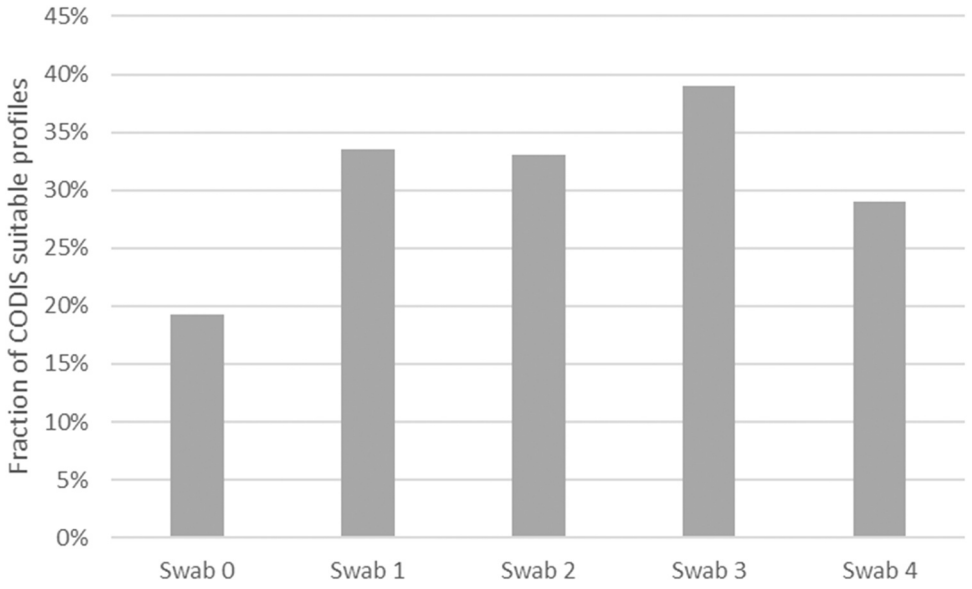

Fig. 3. Fraction of CODIS suitable DNA profiles generated from "touch" DNA traces.

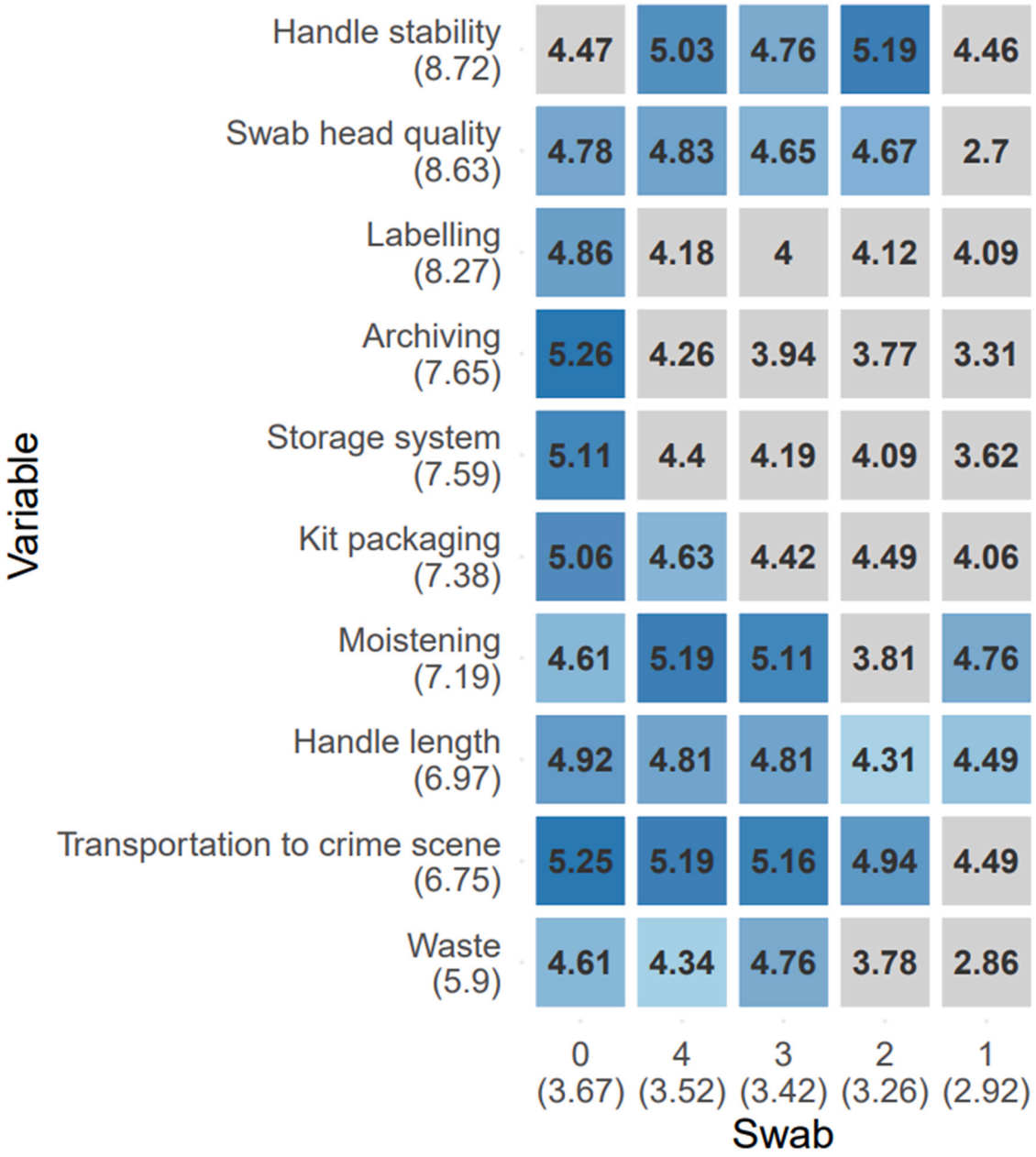

Fig. 4. Global scores for swab types and for each survey item. For each swab type and survey item combination, the mean grade value is reported. The intensity of the blue tile colour is proportional to this value and is greyed out whenever the swab type was not part of the highest significance group (letter a in Fig. 5) for a given survey item. Values between parentheses below survey item names correspond to the average weight given to this item by participants. Values between parentheses below swab names correspond to the swab global score, which is the mean grade weighted by the average item weight.

do not have the effect of soaking the sample into the swab and surface adherence e.g., by electrostatic effects becomes more important. However, all of our swabs were moistened. Dry swabbing is not done by our service. Therefore, we consider our dilution series as a sufficient proxy for the assessment of extraction efficiency from "touch" DNA samples.

4.2. Casework samples – DNA typing success

The sampling of "touch" DNA with all the new swabs tested led to more CODIS suitable DNA profiles than the sampling with Swab 0. (Fig. 3). The success rate of 19.3% for Swab 0 is in line with the 21.7% profiling success rate for this swab type over the entire years 2021 and 2022, including over 8′000 crime scene samples, 87% of which were "touch" DNA samples.

When ranking the Swabs according to performance, as measured by the percentage of successfully established database suitable profiles, Swab 3 performed best, followed by swab 1. In contrast, the current routine swabs (Swab 0) should not be used anymore. These results are consistent with the findings by the Institute of Forensic Medicine in Basel, published during the implementation of this project [17].

Of the samples taken with Swab 0, 23% were collected with double swab technique, compared to 0% for all the other swabs. If we acknowledge that double swabbing is slightly more efficient than single swabbing [25], we could expect an even more pronounced underperformance of Swab 0 if all traces were sampled with just one swab.

We could imagine that the handiness of a certain swab type e.g., its handle length or flexibility, could affect the likelihood of sample

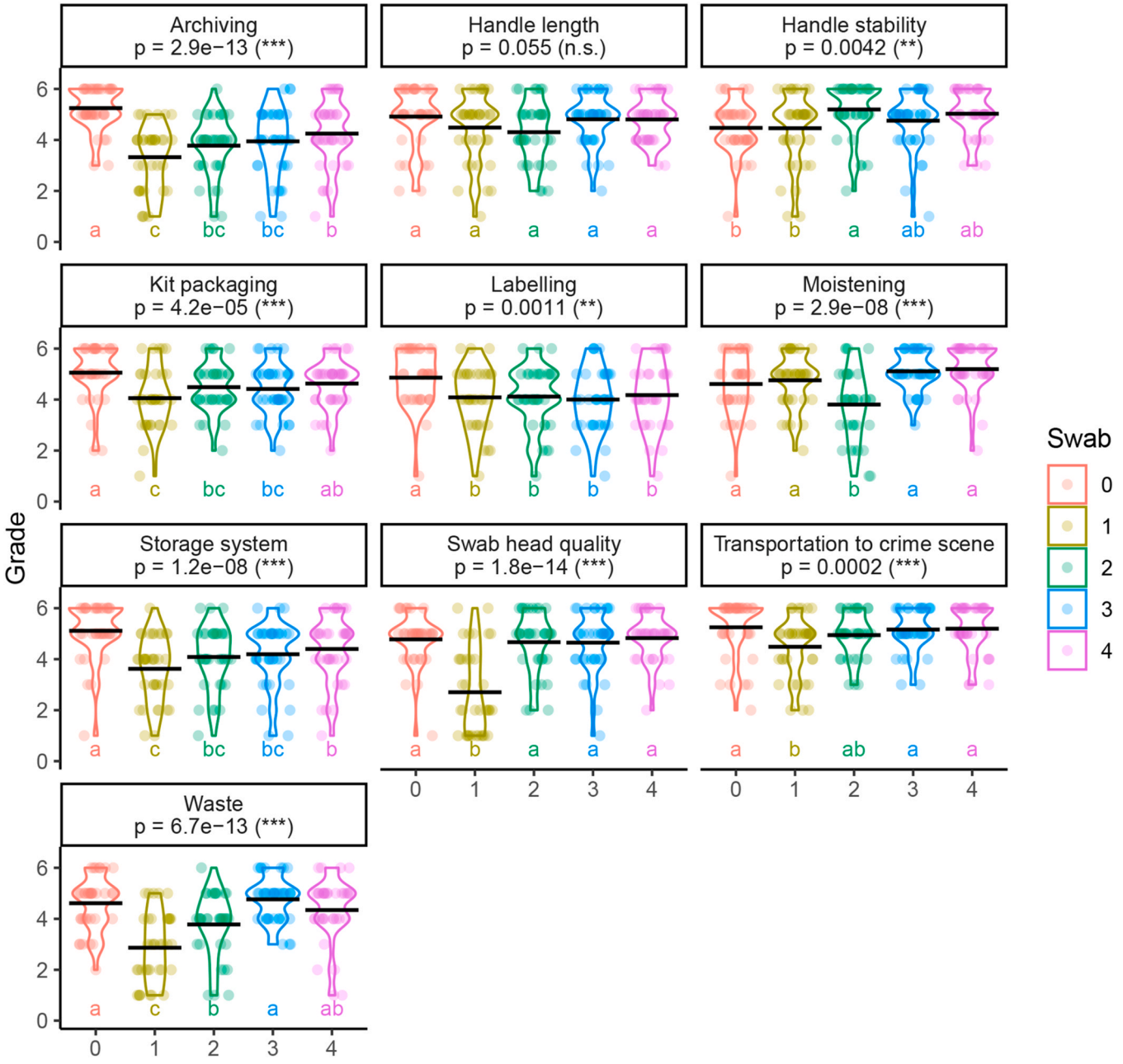

Fig. 5. Differences between swab types for each survey item. In each panel corresponding to survey items, a violin plot shows the distributions of grades (y axis) per swab type (x axis and colours). Individual grades are represented by dots and the black horizontal bar shows the mean value. Below the panel titles, the p-value (p) indicates whether there is a significant effect of the "Swab" variable, based on a mixed-effects linear model. Letters corresponding to significance groups based on a post-hoc procedure are reported below each distribution.

contamination. However, during the test period, we observed only three contaminations by crime scene investigators and none by lab staff. All three contaminations were attributed to different staff members. Two occurred while using Swab 3 and one while using Swab 0. Given the low overall number of contaminations, it is not possible to draw any conclusion about whether one swab is more or less susceptible to contamination than another.

4.3. Staff survey, problems encountered and individual feedback

The subjective feeling of the staff in choosing the swab and the evaluation with weighting of the relative importance of the individual criteria appears to follow the same trend.

If only the subjective result is taken into account, the Swab 4 in particular would be chosen by staff, but many staff would like to stay with the current swab (Swab 0). It could be argued that humans tend to stick to procedures they already know and this is what puts the Swab 0 in second position in the subjective voting, despite of the tedious packaging procedure (manually folding a cardboard box) for this swab type.

However, when looking at the objectivized results, in fact Swab 0 would even be chosen in first place, followed by Swab 4 and 3. The differences between the swabs become less pronounced though, when looking at the objectivized evaluation results, compared to subjective choice.

In both cases, subjective and objectivized evaluation, neither Swab 2 nor Swab 1 would be chosen by the staff. Together with the quality problems found with Swab 1 (loss of the stick), it seems that

this swab should not be chosen, even if it showed very efficient DNA extraction and the second highest DNA typing success rate. Finally, although the order for Swab 1 went smoothly, the only supplier we know of is located in the USA what makes delivery and payment more complicated.

Exception made for the handle stability and the swab head quality, when looking at which points are most important for the staff, the labelling, the archiving, the storage system and the kit-packaging stand out the most. These four criteria are actually less dependent on the swabs themselves, but directly on the wrapping system chosen for this test. Looking at Fig. 4, we can also see that the current swab performed best in those four criteria. Therefore, its ranking in first place might actually be due not to the swab itself, but rather to the well-established procedures around its use. However, when no kit dependent criteria are taken into account, the ranking of the swabs does not change (data not shown). We would nevertheless expect that if the kits were improved, it would change the subjective voting on the swabs.

When selecting the swabs for this test, we believed that one crucial factor for the handiness of a certain swab would be the handle length. Some staff members also explicitly mentioned that the found short handles to be inconvenient for sampling. We were therefore surprised to see, that the evaluation of handle length is the only one that is not significantly different between swabs (Fig. 5) and that this criterion seems not to be priority (criterion placed 8th out of 10).

Another aspect that was deemed important by us, when designing this study was ecological sustainability. With the exception of Swab 0, made of wood and cotton, all swabs involve more or less plastic ware (Fig. 1). However, the amount of waste produced by the use of several thousand swabs per year, even though not entirely irrelevant, with a weight of 5.9, appeared clearly to be the least important aspect in this evaluation. This also argues for a very results-oriented assessment by our crime scene investigators.

It has been demonstrated that different operators achieve significantly different results when sampling for DNA [17]. Such differences are most likely due to differences in handling the sampling device. We must therefore assume that user-friendliness or handiness of a swab also has a direct effect on the DNA result and that it should thus be considered.

5. Conclusion

The testing of a large number of swabs in real case scenarios and the survey filled in by 42 staff members, who regularly work with this type of equipment, made it possible to assess not only the sampling efficiency and the DNA extraction efficiency of the tested swabs, but also important aspects of handling.

From our results, for using them in combination with the PrepFiler Express™ DNA extraction protocol, we can clearly rule out ForensiX 9021040 swabs (Swab 0), because of their bad performance. The performance of the other four swabs was comparable to each other. However, the survey demonstrated that the handling of Bode Securswab 2 (Swab 1) and Copan 4N6 FLOQSwabs® (Swab 2) was not sufficiently appreciated by the staff.

For us, the final conclusion from the present study is that we will switch to ForensiX 9022015 (Swab 3), if their continuous supply can be guaranteed in the near future, or to Sarstedt 80.629 swabs (Swab 4).

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CRediT authorship contribution statement

Conceptualization: **D.C., C.Z.**; Formal analysis: **A.G., D.C., M.Z.**; Investigation: **D.C., M.Z.**; Resources: **C.Z.**; Writing – original draft: **D.C., A.G., M.Z.**; Writing – review & editing: **M.Z.**; Project administration: **D.C.**, Supervision: **D.C., C.Z.**

Declaration of Competing Interest

The authors declare no conflict of interest.

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