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PII: S1872-4973(18)30636-7
DOI: https://doi.org/doi:10.1016/j.fsigen.2019.06.014
Reference: FSIGEN 2113

To appear in: *Forensic Science International: Genetics*

Received date: 6 December 2018
Revised date: 18 May 2019
Accepted date: 18 June 2019

Please cite this article as: Jennifer Comte, Simon Baechler, Joelle Gervaix, Eric Lock, Marie-Pierre Milon, Olivier Delémont, Vincent Castella, Touch DNA collection - Performance of four different swabs, (2019), https://doi.org/10.1016/j.fsigen.2019.06.014

Highlights

- A collaborative study conducted by three police forensic units, a DNA laboratory, and a forensic academic institute about the performance of four different swabs for "touch" DNA collection.
- Experiments undertaken in controlled and quasi-operational conditions.
- From a practical and analytical point of view, COPAN 4N6FLOQSwabs™ [Genetics] presented the best overall performance.
- DNA deposited onto COPAN 4N6FLOQSwabs™ [Crime scene] became severely degraded after a room temperature storage period exceeding three-months.

Touch DNA collection - Performance of four different swabs

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Abstract

A collaborative study conducted by three police forensic units, a DNA laboratory, and a forensic academic institute was undertaken in order to compare the performance of four different swabs in controlled and quasi-operational conditions. For this purpose, a reference swab (Prionics cardboard evidence collection kit) currently used within the police forensic units and 3 challenger swabs (COPAN 4N6FLOQSwabs™ (Genetics variety), Puritan FAB-MINI-AP and Sarstedt Forensic Swab) were used for collecting DNA traces from previously used items (referred as "touch DNA" in this article) including on 60 collars, 60 screwdrivers and 60 steering wheels obtained from volunteers. For each comparison, the surface considered was divided into two equal components ; one was sampled with the reference swab and the other with one of the three challenger swabs. This lead to a total of 360 samples. Conclusions were consistent within the

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Abstract

A collaborative study conducted by three police forensic units, a DNA laboratory, and a forensic academic institute was undertaken in order to compare the performance of four different swabs in controlled and quasi-operational conditions. For this purpose, a reference swab (Prionics cardboard evidence collection kit) currently used within the police forensic units and 3 challenger swabs (COPAN 4N6FLOQSwabs™ (Genetics variety), Puritan FAB-MINI-AP and Sarstedt Forensic Swab) were used for collecting DNA traces from previously used items (referred as "touch DNA" in this article) including on 60 collars, 60 screwdrivers and 60 steering wheels obtained from volunteers. For each comparison, the surface considered was divided into two equal components ; one was sampled with the reference swab and the other with one of the three challenger swabs. This lead to a total of 360 samples. Conclusions were consistent within the four operational partners. From a practical point of view, the COPAN 4N6FLOQSwabs™ (Genetics variety) was judged the most convenient to use. Furthermore, it allowed the recovery of significantly more DNA from collars (0.65 vs 0.13 ng/uL) and steering wheels (2.82 vs 1.77 ng/uL), and a similar amount of DNA from screwdrivers (0.032 vs 0.026 ng/uL) compared with the Prionics reference swab. The two other challenger swabs provided results that were not significantly different from the reference swab, except for the Puritan swab, whose performance was significantly lower for steering wheels (0.37 vs 0.58 ng/uL). As part of a conservation study, 50 uL of a blood dilution (1/4 with PBS) was deposited on a total of 105 COPAN (Genetics and Crime Scene varieties), Prionics and Sarstedt swabs. They were stored within a cupboard at room temperature. The integrity of the recovered DNA was evaluated with NGM SElect™ DNA profiles after different time-spans ranging from 1 day to 12 months by comparing the height difference of the peaks occurring at the shortest and longest loci, respectively. DNA seemed to remain stable, except when using the COPAN

4N6FLOQSwabs™ treated with an antimicrobial agent (Crime scene variety), which resulted in significant DNA degradation. Following these tests, the COPAN 4N6FLOQSwabs™ (Genetics variety), a model with a desiccant, was selected for further testing in fully operational conditions.

Keywords : Touch DNA, Flocked swab, Cotton swab, Sampling, DNA preservation, DNA collection

1. Introduction

In order to maximize the chance of obtaining an informative DNA profile from a sample collected on a crime scene or in the laboratory, it is important to use a device able to provide an efficient and selective collection of traces. This to preserve their integrity by limiting subsequent pollution and degradation, and to allow an effective recovery of the biological material for DNA analysis. Such considerations imply that successful DNA profile relies not only on the laboratory's analytical process but also on the general sampling procedure used by the police's crime scene examiners or forensic investigators.

Various collection methods exist [1], such as: cutting [2], FTA paper scraping [3], scraping of the surface of interest with wooden applicator stick [4] or sterile scalpel blade [5, 6], taping [3, 5, 7, 8, 9] or vacuum sampling [2, 10] and wet or dry, single or double swabbing [3, 5, 7]. Swabbing is the most versatile method and one of the most frequently used. At least, this is the case within the forensic units involved in this study. Over a number of years, they have been extensively using swabs for DNA collection, both for crime scene investigations and laboratory examinations. Because of the increase in swab types available on the market, the promises of commercial arguments, and the results of various research studies conducted in controlled conditions with several swabs and/or swabbing conditions [11], questions then arose among our institutions as to whether the swabs in use were still suitable, whether they met the actual scientific state of the art, and whether they were the most efficient considering a set of criteria. To address these questions, a collaborative study was conducted. The study's novelty resided in the combination of three critical aspects. 1) While most of the published studies consider blood or saliva dilutions to get a better

control on the deposition of biological material, we considered touch DNA [1] samples because they tend to be the most frequent and the most challenging specimens. Indeed, 85% of the crime scene specimens sent to the DNA laboratory of Lausanne in 2017 (N=13'463) were touch DNA specimens. 2) The study is based on the joint endeavour of partners with complementary perspectives: three operational police forensic units (attached to the state police of Geneva, Neuchâtel and Vaud in Switzerland), the DNA laboratory working with these police departments and a forensic academic institution. 3) The study was built around a progressive and adaptable structure of successive steps. This structure started with a series of experiments undertaken in controlled conditions, and evolved into a fully operational campaign (currently in progress) which aims to assess the use of the selected swab in real conditions, i.e. during the daily activities of staff in partner institutions over a period of several months.

This paper reports the findings of the first steps of the experimental design. The swab currently used by the police forensic units, the *reference swab*, is compared in quasi-operational conditions against three alternative swabs, the *challenger swabs*. Using this "duelling" procedure, combined with a DNA preservation test, the purpose of this study was to select a convenient swab, both for the police forensic units and for the laboratory, that maximizes DNA recovery from touch samples and preserves DNA when stored at room temperature.

2. Materials and Methods

The contributions to this study were divided as follows: all five partners collaborated in the design of the study; the three police units and the laboratory carried out the experiment; and the laboratory analyzed the samples and performed statistical analysis with the support of the forensic institute.

1. The term "touch DNA" was chosen because items selected in this study are used in direct skin contact (except in case of wearing gloves). The background history and actions surrounding the items sampled are not known, and therefore, neither is the nature of the biological material collected. Low levels of DNA could also come from bodily fluids.

2.1. Selection of challenger swabs

Currently, the three police forensic units are using the same evidence collection kit produced by Prionics, consisting of cotton swabs, sterile water ampoules, cardboard boxing and adhesive seals. This kit was routinely used for many years and was therefore considered as the *reference swab* for this study. Together, the three police forensic units, the forensic academic institution and the DNA laboratory determined practical and analytical criteria for the choice of commercially available *challenger swabs*. In order to minimize the potential risk of pollution, exacerbated by an open-air drying step, only devices allowing the swab packaging to be closed immediately upon collection were considered. We evaluate different enclosed drying systems in order to assess DNA preservation. Furthermore, since swab components (glue, fibers, shaft,...) might interfere with presumptive tests for the presence of biological fluids [12] or the DNA extraction process [13], preliminary tests were undertaken to verify the absence of negative interaction between the selected swabs and the procedures used within the different services (unpublished results). Based on these preliminary tests, three *challenger swabs* were selected for further testing: the Sarstedt Forensic swab (Sarstedt AG, Germany), the Puritan FAB-MINI-AP swab (Puritan Medical Products, USA) and the COPAN 4N6FLOQSwabs™ (Genetics and Crime scene varieties), Copan Italia S.p.A., Italy). Technical characteristics of each swab as well as each entity requirements are presented in Tables A.1 and A.2. Shaft characteristics, fiber types and layouts, and drying systems were their main functional differences. The distinction between the two COPAN versions is also provided in section 2.3.2.

2.2. Substrates

The nature of the substrate certainly influences the chance of obtaining an informative DNA profile from touch DNA specimens [11, 14, 15, 16, 17]. Consequently, for the first part of our comparative study, three substrates having well-contrasted characteristics and being routinely used for DNA sampling by police forensic units were chosen: cover-less steering wheels of different materials (leather, hard plastic, imitation leather), screwdriver handles, and shirt/t-shirt collars worn for at least one day. Members from the three police forensic units and the DNA laboratory volunteered their personal belongings to be sampled. Thus, DNA deposits were the result of everyday use and not simulated in the lab. The chosen surfaces had the

particularity of either being smooth and non-porous (screwdrivers), rough and non-porous (steering wheels, screwdrivers) or absorbent (collars). These also offered different area sizes for sampling. The study focused on the collection and release capacities of the swabs only in terms of DNA amount and not in terms of profile characterization. Since the conditions were not controlled, it is likely that DNA mixtures would occur.

2.3. Sampling and analytical procedures

2.3.1. First Part: Comparison between reference swab and challengers

The technical characteristics of the swabs, such as the type and layout of the fibers as well as the size of the head are likely to influence collection and release of biological material efficiency. The tested devices are the following: COPAN 4N6FLOQSwabs™ Genetics, Puritan FAB-MINI-AP, Sarstedt Forensic, and the reference Prionics evidence collection kit. For each of the four services, a single person was designated as the operator that carried out the experiments. This led to the production of four independent sets of results and allowed us to consider the potential influence of the operator on the collection efficiency of each swab.

DNA collection was performed under real-world conditions, following a "duelling" procedure where each surface was split into two equal parts in order to make paired comparisons between the reference swab and one of the challenger swabs. For steering wheels, the two halves (left and right sides) were sampled randomly and alternately to account for possible discrepancies in DNA deposit (potential differences could be due to either the use of the right hand to shift gears or difference in shedding between the right and the left hand). One half of the surface was swabbed with the reference swab, while the other half was swabbed with one of the challenger swabs. This was repeated 5 times per substrate (3) and per challenger swab (3) for a total of 45 sample pairs per operator. This led to the collection of 90 samples per service for a total of 360 samples.

Following manufacturer's recommendations, one drop of water was used to moisten the COPAN swabs when collecting touch DNA from screwdrivers and steering wheels, and no water was used for the collars (fabric). Neither of the three other manufacturer provide moistening recommendations for forensic cases. At the time, the routine protocol for the Prionics swab was to moisten a part of the swab with approximately three drops of sterile water provided in the kit (Table A.1). Following this protocol, the moistened part was rubbed or rolled on the surface, followed by the entire swab head in order

to collect the sample. Operators proceeded as they usually would without specific instructions on how to rub or roll the swab. The same moistening technique was applied for the Sarstedt swab because it presents the same head thickness as the reference swab. Concerning the Puritan swab, only one drop was used because of its low thickness.

Steering wheels were sampled in situ. For shirt collars and screwdrivers, each service chose a single date for their volunteers to bring their personal belongings to the sampling room of their service. Volunteers handed their belongings either in a paper bag or without any particular packaging. The designated operator for each service performed the sampling of each item. After collection, the packaging for the swabs was immediately closed. The samples were brought to the DNA laboratory. A period of 3 days was always respected between the sampling and the analysis. During this time, samples were stored at room temperature in a cupboard. This experimental design allowed for the evaluation of the relative performance and the practicality of the four swabs considered for collecting touch DNA on different substrates.

2.3.2. Second Part: DNA preservation

Following operational procedures in place within the partner institutions, samples are routinely stored at room temperature (RT) before being analyzed. Although RT storage is convenient since it does not require cooling devices, studies have shown that DNA damage may already occur a few hours after collection when swabs are stored wet [18, 19, 20]. This could be problematic since swabs can be stored weeks or months within police forces and/or the DNA laboratory before being processed. It is therefore essential to use collection swabs allowing the proper preservation of the DNA under actual storage conditions. Therefore, swabs considered in this study were selected because they are designed for conserving DNA at RT without any prior drying step. In order to achieve this goal, some models are supplied with a cardboard box (Prionics swabs) or a plastic tube with a permeable membrane (Sarstedt and Puritan) enabling the moisture to evaporate. COPAN 4N6FLOQSwabs™ are available in two versions: the "Genetics" variety has a desiccant within the cap of the plastic tube to absorb residual water. Whereas the "Crime Scene" variety, also in a plastic tube, has its head treated with an antimicrobial agent. This latter is thought to prevent microorganisms growth and therefore protect DNA. Since the characteristics of the swabs heads are very similar, only the Genetics variety was tested for its capacity to collect touch DNA. However

the two varieties of COPAN swabs were considered for testing preservation of the recovered DNA. Due to its relatively poor performance for collecting touch DNA, the Puritan swab was not considered for the preservation study.

The tested devices were: Prionics with its cardboard box, COPAN with antimicrobial agent, COPAN with a desiccant system, and Sarstedt tube with ventilation membrane (see Table A.1). In order to evaluate the stability of the DNA stored at room temperature, 50 µl of blood from one volunteer, diluted with 1/4 PBS (Sigma Aldrich, Switzerland), was deposited on the swabs heads. A volume of 50 µl of blood correspond to the 3 drops of water that are routinely used by the police forensic units to moisten the reference swabs before trace collection. The boxes and tubes containing the swabs were immediately closed and deposited within a cupboard (door closed) in an air-conditioned room. The mean room temperature was 22±2°C and the mean relative humidity was 35±5%. The time intervals between blood deposition and DNA analysis were of 1 day, 1 and 2 weeks, 1, 3, 6 and 12 months. Triplicates were performed, leading to a total of 105 samples.

2.4. DNA extraction and quantification

COPAN heads were broken off at the breaking point, while the cotton swab heads with part of the shaft were cut below the cotton with sterile scissors. DNA extraction was performed with a PrepFiler™ Automated Forensic DNA Extraction Kit/Microlab STAR Line automated system, co-developed by Applied Biosystems (AB, Foster City, CA) and Hamilton®. Trace items were placed in AutoLys tubes manufactured by Hamilton. Cell lysis was performed on an AutoLys STAR platform (incubation of 60 minutes at 70°C). Incubation temperature and duration for an optimal recovery of DNA from cotton swabs had been determined prior through internal validation. AutoLys tubes are designed with close-fitting outer and inner tubes in addition to a lift-and-lock system that allows centrifugation in order to collect all the liquid absorbed by the cotton/nylon. An ID STARlet platform was used for DNA purification. The PrepFiler™ large volume protocol was followed, which is the routine procedure.

Real time qPCR analysis was performed using the Investigator Quantiplex HYres™ Quantification kit (QIAgen) using a 7500 Real Time PCR system instrument following instructions provided by the supplier with the exception of half reaction volumes being used. DNA samples from

the same substrate were extracted and quantified only once on the same quantification run.

2.5. DNA profiling

For the DNA preservation study, DNA was amplified with the AmpFLSTR™ NGM SElect™ PCR Amplification Kit (Applied Biosystems) using 1 ng of template DNA in a total reaction volume of 25 µl. This kit amplifies 16 STRs markers plus the amelogenin gender-marker, those are labeled with four different fluorochromes. A fifth fluorochrome is used for the 500 LIZ size standard. Amplifications were performed as specified by the manufacturer using 30 PCR-cycles with Veriti thermal cyclers (Thermo Fisher Scientific). For each sample, 1 µl amplicon, 8.5 µl deionized formamide Hi-Di (Applied Biosystems) and 0.5 µl 500 LIZ size standard (Applied Biosystems) were used for capillary electrophoresis with ABI 3500 genetic analyzers (Applied Biosystems) following standard procedures.

2.6. Statistical analysis of quantification data and qualitative analysis of electropherograms

For the collection and release capacities study (2.3.1), the ratios between the concentrations of DNA released by the challenger swabs and the reference swabs were calculated. A Wilcoxon Signed Rank test was carried out to evaluate the significance levels between the DNA concentrations detected. A three-way ANOVA test was applied to find which factors were more relevant among swabs, operators and substrates to influence touch DNA concentration, taking into account their possible interactions. Those statistical analyses were performed with R software.

For the DNA preservation study (2.3.2), electropherograms were analyzed with GeneMapper™ ID v3.2.1 software (Applied Biosystems). Peak heights (RFU) were exported along with the allelic designations (Fig. 1). The longer DNA fragments are more prone to degradation compared to the shorter ones. Therefore, a ratio was calculated by dividing the sum of the heights of the 2 alleles occurring at the longest STR loci by the sum of the heights of the 2 alleles occurring at the shortest loci within each of the four color channels: D2S1338/D10S1248, D18S51/D8S1179, FGA/D22S1045 and SE33/D2S441 (see Table 1). This ratio was defined as the Integrity Index (INTI). INTI was averaged across the four color channels. Finally, mean values and standard deviations were obtained using the 3 replicates for each swab and each period

of time elapsed between blood deposition and DNA analysis. INTI varies from 0 to 1 and is a measure of non-degraded DNA. When INTI=0, the alleles occurring at the longest STR loci are completely missing. Conversely, when INTI=1 the height of the alleles is not lower for the longest fragments and there is no sign of DNA degradation.

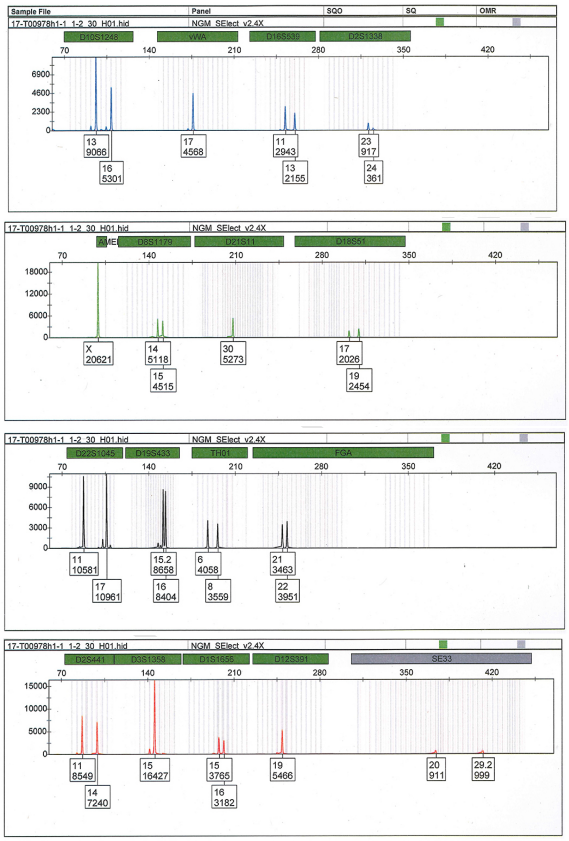

Figure 1 : The volunteer DNA profile is heterozygous at the loci used for calculating the Integrity Index. The mean sizes of the alleles occurring at the shortest and longest DNA fragments are 110 and 318 bp respectively. The DNA profile corresponds to the COPAN Crime scene swab after 12 months storage at room temperature.

Channel EPG	Peak heights	Integrity Index (INTI)
Blue	(917+361)/(9066+5301)	0.09
Green	(2026+2454)/(5118+4515)	0.47
Black	(3463+3951)/(10581+10961)	0.34
Red	(911+999)/(8549+7240)	0.12
Mean of 4 channels		0.25
Mean of the 3 replicates		0.20 ± 0.10

Table 1 : The Integrity Index is calculated as the 4 channel mean ratio of the relative fluorescent unit (RFU) heights of the 2 longest alleles over the RFU heights of the 2 shortest alleles. Means and standard deviations were obtained from 3 replicates. The values shown correspond to the COPAN Crime scene swabs after being stored 12 months at room temperature.

3. Results

3.1. First Part: Comparison between reference swab and challengers

3.1.1. General comments on swab practicality

During the trials, some important practical points were observed (see Table A.2). None of the COPAN swab heads broke up during the sampling and the shaft offered an appreciated combination of flexibility and rigidity. The breaking point of the head was appreciated by the laboratory as it facilitated the cutting of swabs. However, if too much pressure is applied on the substrate during trace collection, the shaft could break and cause the swab head to be catapulted, with a risk of contamination. Regarding the Sarstedt swab, cotton fibers seemed to be tighter and did not absorb sterile water as well as the others. Also, its shaft was judged to be slightly too pliable. Concerning the Puritan swab, both the opening and the closure of the tube were considered unsafe and presented a potential risk for contamination because the shaft is not attached to the cap of the tube. There was also not enough room for labeling/writing on this tube. However, the mini-tip allowed for reaching into small or difficult access areas, like seams. From a practical point of view, the COPAN swab was rated as the best by the four operators.

3.1.2. Collars

Figure 2 and Table A.3 show the range of collected DNA amounts for the 20 sample pairs and the results from the sampling comparison. The mean value for total DNA concentration of the COPAN swab (Cop) was five fold that of the Prionics swab (Pri) (0.65 ng/µl vs 0.13ng/µl) (Table

A.3). This difference was significant (Wilcoxon p-value <0.05). For this substrate, the COPAN swab performed better than the Prionics swab for all operators and each of the trials. Mean values were similar between the Sarstedt swab (Sar) and the Prionics swab (0.11 ng/µl vs 0.09 ng/µl) and between the Puritan swab (Pur) and the Prionics swab (0.05 ng/µl vs 0.06 ng/µl). Concentrations were not significantly different between the reference and the two other challengers (Wilcoxon p-value >0.05).

3.1.3. Screwdrivers

Figure 3 shows the results from the sampling comparison and the range of collected DNA amounts for the 20 sample pairs. Total DNA concentration mean was similar for each paired comparison (Table A.4). No significant differences were observed (p-value >0.05).

3.1.4. Steering wheels

Figure 4 and Table A.5 show the results from the sampling comparison and the range of collected DNA amounts for the 20 sample pairs. The mean DNA concentration was two-fold higher for the COPAN swab compared to the Prionics swab (2.82 ng/µl vs 1.77 ng/µl), lower for the Puritan swab compared to the Prionics swab (0.37 ng/µl vs 0.58 ng/µl) and similar between the Sarstedt swab and the Prionics swab (1.29 ng/µl vs 1.39 ng/µl). Differences were significant for the COPAN swab (Wilcoxon p-value <0.05 (with or without the outlier) and for the Puritan swab (Wilcoxon p-value <0.05) but not significant for the Sarstedt Swab (p-value >0.05).

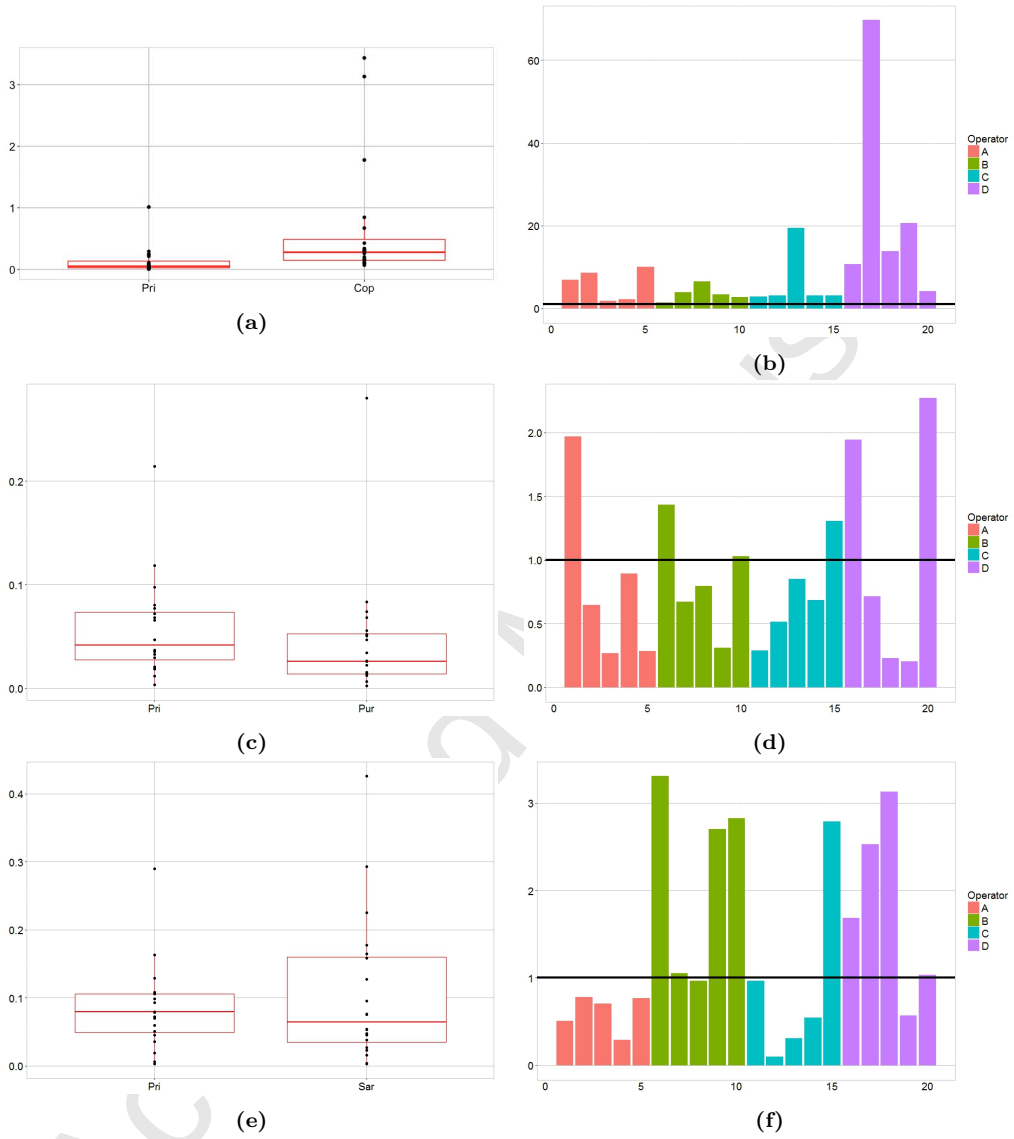

Figure 2 : Comparison between challenger swabs (Cop, Pur, Sar) and reference swab (Pri) on collars. (a,c,e) Boxplot distribution. Range of biological material amount (ng/µl) collected with each swab, all operators combined. (b,d,f) Ratio [Challenger Swab]/[Reference Swab] (ng/µl) for each operator (A to D) on each trial. A ratio of 1 indicates both swabs performed equally well (horizontal line). Values above this line indicate that more biological material was collected with the Cop (b), Pur (d) or Sar (f) respectively.

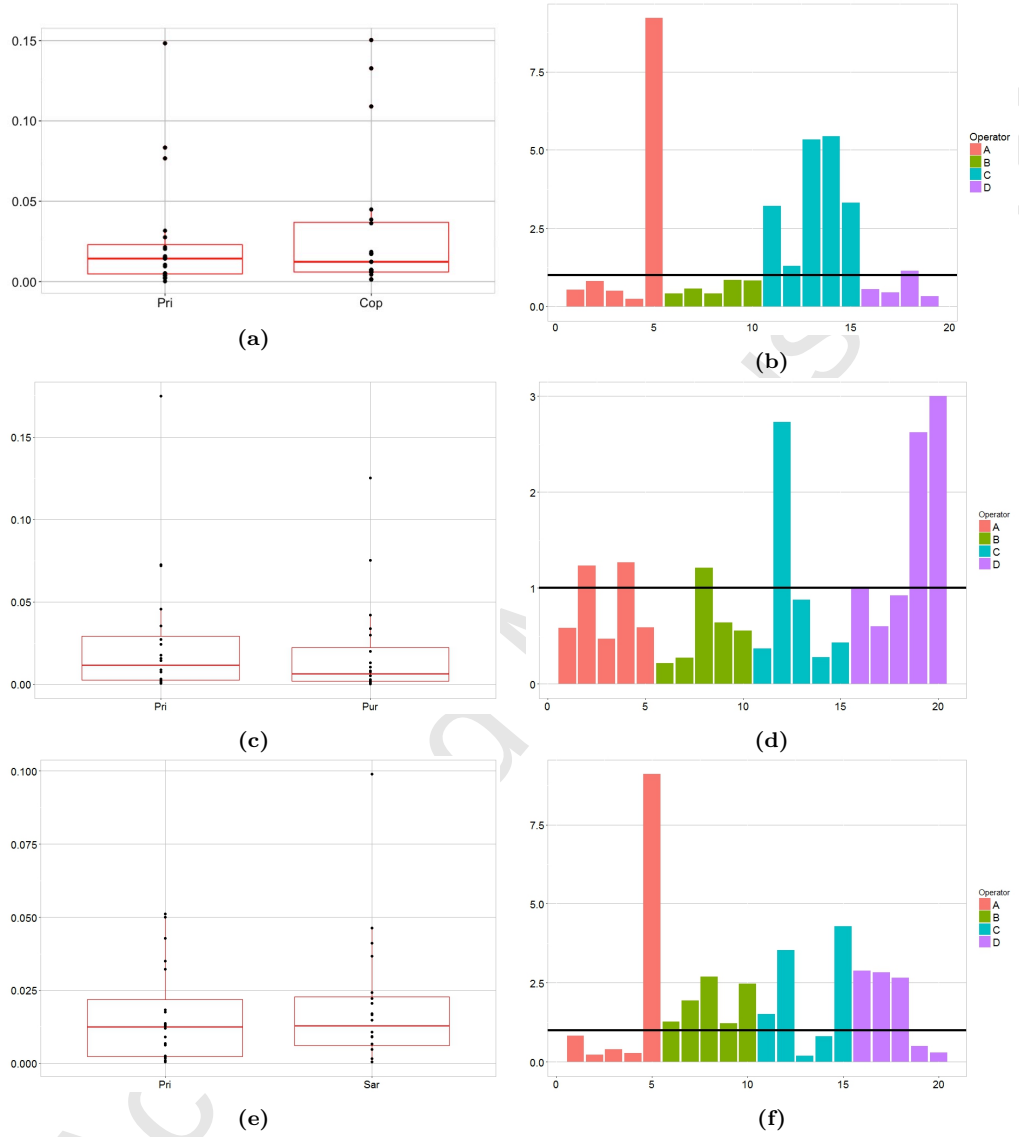

Figure 3 : Comparison between challenger swabs (Cop, Pur, Sar) and reference swab (Pri) on screwdrivers. (a,c,e) Boxplot distribution. Range of biological material amount (ng/µl) collected with each swab, all operators combined. (b,d,f) Ratio [Challenger swab]/[Reference] (ng/µl) for each operator (A to D) on each trial. A ratio of 1 indicates both swabs performed equally well (horizontal line). Values above this line indicate that more biological material was collected with the Cop (b), Pur (d) or Sar (f) respectively. (b) The last trial for operator D is removed for graphical representation. The ratio value is: 92.5.

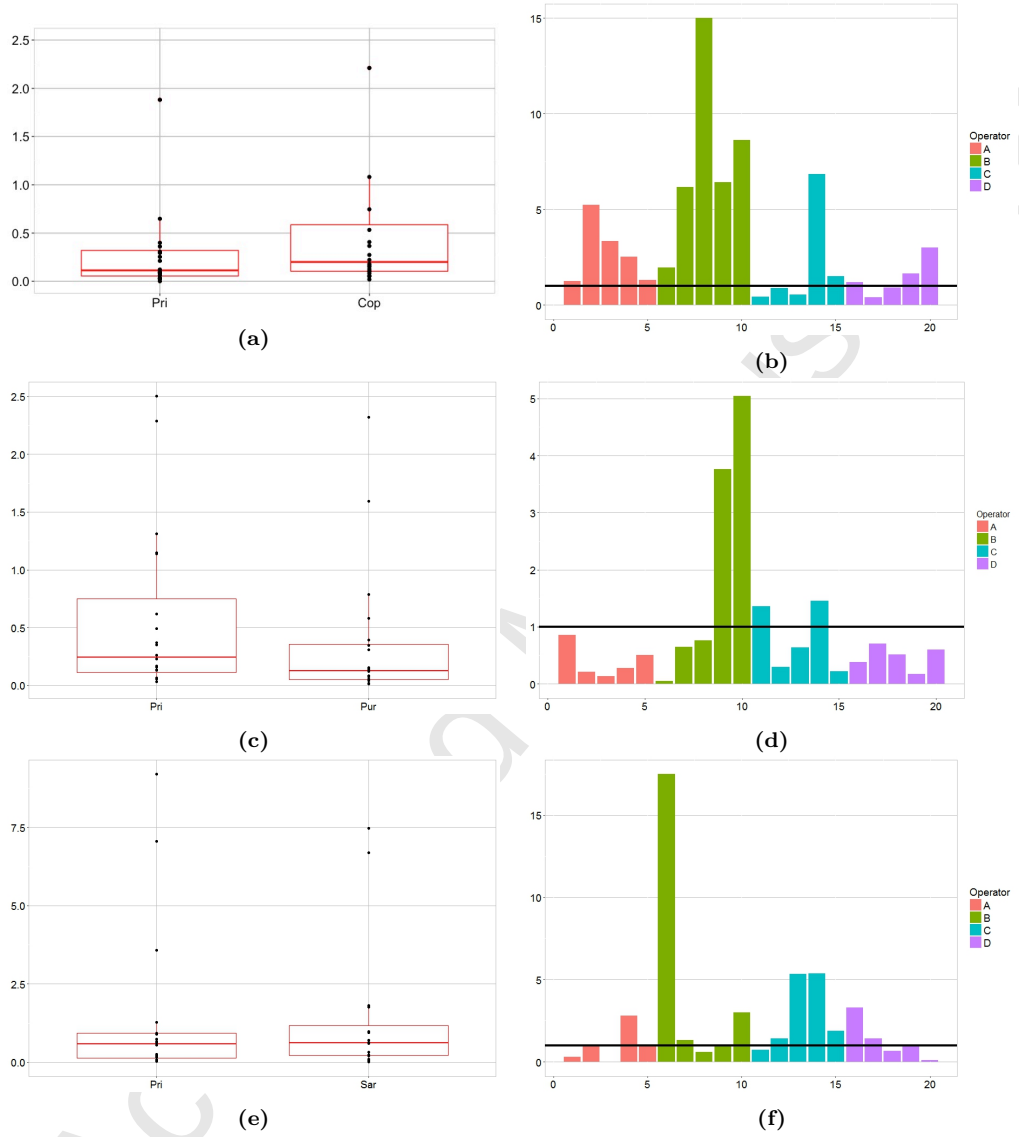

Figure 4 : Comparison between challenger swabs (Cop, Pur, Sar) and reference swab (Pri) on steering wheels. (a,c,e) Boxplot distribution. Range of biological material amount (ng/µl) collected with each swab, all operators combined. (a) 2 pairs are removed for graphical representation (Cop = 4.42 ng/µl / Pri = 0.65 ng/µl and Cop = 45.16ng/µl / Pri = 30.37ng/µl. (b,d,f) Ratio [Challenger swab]/[Reference] (ng/µl) for each operator (A to D) on each trial. A ratio of 1 indicates both swabs performed equally well (horizontal line). Values above this line indicate that more biological material was collected with the Cop (b), Pur (d) or Sar (f) respectively. (f) Ratio value of 0.003 for the third trial of operator A.

3.2. Second Part: DNA preservation

DNA profile quality from blood dilutions deposited on the different swabs up to one month storage remained quite stable: no significant difference appeared among swabs (Fig 5), with integrity indexes (INTI) ranging from 1.02 (Sarstedt, 1 day) to 0.88 (COPAN Genetics, 1 month). All but one swab followed a common trend across time. From 3 to 12 months, INTI decreased and varied between 0.96 (COPAN Genetics, 6 months) and 0.77 (Sarstedt, 12 months). The notable exception was the COPAN Crime scene, having a significantly lower INTI after 3 months (0.72), 6 months (0.44) and 12 months (0.20).

Full DNA profiles were obtained for every sample considered in the degradation study, independently from the swab, the storage time and the integrity index.

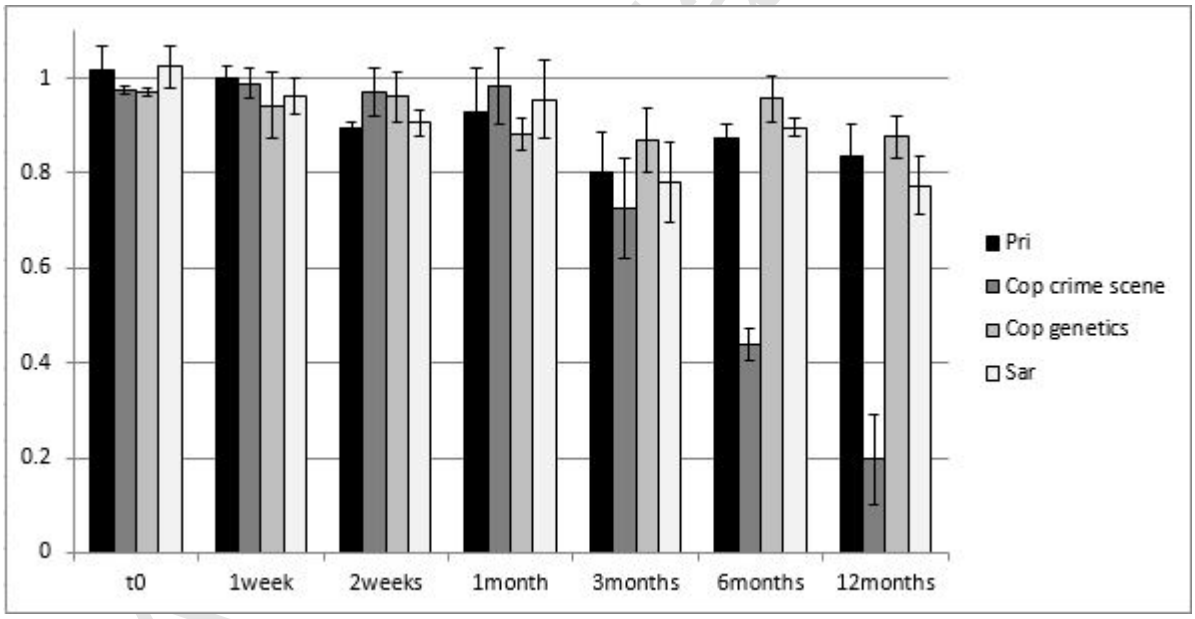

Figure 5 : Integrity Index (INTI), a measure of non-degraded DNA, estimated after storing the 4 swab brands at room temperature for 1 day, 1 and 2 weeks, 1, 3, 6 and 12 months. After 12 months, DNA collected with the COPAN crime scene swab appears to be particularly degraded. At t=0 the mean INTI values are greater than 1 for Prionics and Sarstedt swabs. This indicates that the height of the alleles was higher for the longer fragments. This is due to the amplification variability which can occur with fresh blood samples.

4. Discussion

Since 2000, the majority of police forensic units and DNA laboratories in Switzerland have been using Prionics cotton swabs moistened with sterile water to collect biological traces (blood, sperm, saliva, touch DNA) at crime scenes or in the lab. Over the last decades, sampling procedures (single or double swabs) and extraction processes have been progressing, increasing the sensitivity of DNA analyses and allowing the consideration of traces with very small amounts of DNA. As a result, the types of collected specimens changed: the last five years, touch DNA accounted for at least 85% of the traces submitted to the forensic genetics laboratory of Lausanne, Switzerland. In parallel, probably because of the many hits and operational successes achieved using Prionics swabs over the years, the use of this evidence collection kit was not questioned by practitioners. This was despite studies indicating that cotton swabs could trap (i.e. not release) some of the biological material collected or could interact with the DNA extraction process, resulting in a loss of material for the DNA analysis [13, 21, 22]. In addition, published research has shown different DNA yield because of swab models variable performance [11]. We then ask ourselves whether or not the swab in use was the best.

To our knowledge, no published study has examined the selection of a proper device for improving the collection and preservation of touch DNA in real operational conditions. This may be because of the complex nature of touch DNA, which consists mostly of sloughed, enucleated keratinocytes [23, 24] and extracellular [25], partially degraded DNA derived from apoptotic epithelial cells, sebaceous [26] or sweat glands [27]. For this reason, it is complicated to identify which of the following variables (or their combinations) have a significant influence on DNA collection [1, 21, 28, 29, 30] : the swab head size, the layout and type of fibers, the static electricity of a dry swab, the use of a solvent to moisten the swab and consequently the substrate, the operator or the drying system. In the first part of this study, the relative and global performance as well as the practicality of four swabs considered for collecting touch DNA on three different substrates was assessed. The COPAN 4N6FLOQSwabs™ (Genetics variety) presented the best overall performance. It performed better than the Prionics swab for collecting touch DNA on shirt/t-shirt collars and steering wheels. On the other hand, on screwdrivers handles, items with the least amount of DNA, it did not show a significant

advantage. Conversely, Puritan and Sarstedt swabs presented similar or poorer performance in comparison to the Prionics swab across the various substrates.

Among the swab, the operator and the surface, a three way ANOVA test determined that only the swab was a significant factor (p-value <0.05) with regards to the amount of DNA collected. The combination operator-swab is close to being significant with a p-value of 0.055. In some situations, such as those presented in Figures 2(f) and 3(b), the challenger swab performance seemed to vary depending on the operator who collected the sample, suggesting that sampling methods and their effect should require further detailed investigations in order to improve DNA collection with the chosen device. In the present study, operators were asked to use swabs as they do routinely in casework to remain as close as possible to real operational conditions. All other factors or combinations have a p-value >0.1.

Since touch DNA specimens often contain low amounts of DNA, efficient preservation is essential. The institutions collaborating on this study routinely store DNA samples at room temperature (RT), protected from light. RT storage is convenient because it does not require cooling systems such as freezers or cold rooms, and the temperature is easily maintained when samples are transported. However, RT storage requires the swab to be dry to avoid DNA degradation. Leaving the packaging open until the swab is dry could be a solution, but this requires a wait of several hours (eg. [20]) and the risk of mix-up and pollution is non-negligible when several specimens are processed together. Drying systems have been designed that allow the device to be closed immediately upon collection. DNA stability data, according to the characteristics of the packaging of the swab, are available [18, 19, 20, 31, 32, 33]. But it is difficult to compare the different studies because no consensus exists among them regarding the measurement of DNA degradation. Some authors simply looked at the evolution of DNA concentration (e.g. [2, 18]), while others monitored the evolution of the proportion of alleles detected. Recently, several DNA quantification kits have included degradation indexes (DI). However, the size of the DNA fragments targeted as well as the calculation of DI differs between kits [34]. As DNA profiles represent the final outcome of forensic DNA analyses, we choose to use an integrity index (INTI) which is calculated from electropherograms. Degradation causes a "ski slope pattern" with a decrease of the peak heights according to increasing DNA fragment size. INTI reflects this slope and is easy to understand since it

varies from 0 to 1. Our findings showed that DNA was relatively stable during the first year when swab packaging allowed moisture elimination. Either through the permeability of the packaging (Sarstedt and Prionics swabs) or by the presence of a desiccant (COPAN genetics variety swabs). In contrast, DNA collected with COPAN Crime scene swabs became severely degraded after a storage period exceeding three-months. Interestingly, this swab packaging does not allow for the release of humidity, but its head is treated with an antimicrobial agent to prevent the growth of microorganisms. Such degradation would probably not affect DNA rich specimens. However, when analyzing small amount of DNA such as touch DNA specimens, it is likely that such degradation will generate partial DNA profiles with missing information mainly at the longest STR loci. As a potential solution, freezing the swabs could slow down this detrimental process but requires significant logistical adaptations in practice.

5. Conclusions

Forensic scientists and criminal justice stakeholders wish to achieve the best performance in DNA profiling. This aim encompasses several dimensions; DNA profiling depends on interdependent processes that are in the hands of different partners, with their own constraints and needs. Most of the time, these processes are considered separately in research work and practice. Consequently, potential interactions are neglected when trying to optimize one of the individual components. For instance, it is useless to select a swab that collects a lot of DNA if this material is then degraded and lost during storage or DNA extraction. Therefore, selecting the "best" device to collect biological traces requires more than mere analytic comparisons in lab conditions.

Within the present study, a collaborative approach bringing together several police forensic units with a DNA laboratory and a forensic academic institute was favoured in order to define a holistic or end-to-end vision of performance. As a first step, common criteria were defined to compare three models of swabs available on the market against the model used routinely for a long time. The collection of biological traces with the swab was considered in combination with Prepfiler extraction and storage of the material collected at room temperature. Comparative tests were conducted in quasi-operational conditions, using touch DNA as well as various

substrates and operators, in order to assess DNA collection, extraction and preservation. Based on the findings of these experiments, the partners decided to engage in performing a follow-up study in fully operational conditions. The COPAN 4N6FLOQSwabs™ (Genetics variety) is now implemented in their everyday practice as their operational collection device. The evolution of touch DNA specimens results will be monitored in order to assess the performance of the COPAN 4N6FLOQSwabs™ (Genetics variety) in comparison to the Prionics swabs in full operational conditions. Our research efforts do not aim to provide every forensic unit and laboratory with a universal collection device. It is a local solution which takes into account several parameters specific to our entities. It is likely that other combinations of the processes tested may provide good results elsewhere. However, we are convinced that findings from the different steps of this project may be useful or inspirational for other practitioners.

Conflict of interest

None.

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AnnexeA. Supplementary data and figures

Supplementary data and figures associated with this article can be found, in the online version.

Swab abbreviation	**Pri**	**Cop**	**Pur**	**Sar**
Full Name	Prionics cardboard evidence collection Kit	COPAN 4N6FLOQSwabs® • Crime Scene • Genetics	Puritan FAB-MINI-AP	Sarstedt Forensic Swab
Reference	9021040	• 3510C • 4504C	28-825 1WCS TT FABUSA	80.629.001
Head composition	Cotton	Nylon	Mini-tip Cotton	Cotton
Arrangement of fibers	Wound	Flocked	Wound	Wound
Shaft composition	Wood	Plastic	Wood	Wood
Shaft length	135 mm	109 mm	129 mm	95 mm
Treatments	sterile & EtO sterilised	DNA free & EtO sterilised	Sterile	EtO sterilised
Storage	Permeable cardboard box (to be folded)	Plastic tube	Plastic tube	Plastic tube
Protection against DNA degradation	natural ventilation outside cardboard box	• antimicrobial chemicals on fibers • desiccant within the cap	air holes covered by a breathable filter on the side of the tube	ventilation membrane on the bottom of the tube
Amount of sterile water* used to moisten	3 drops	1 drop except for clothes to swabed without water	1 drop	3 drops

Table A.1 : Intrinsic characteristics of swabs. * from sterile water vials 0.45ml (ref A32265C Thermo Fisher)

	Pri	Cop	Pur	Sar
Easy to pack after use	+	++	-	++
Absorption of moistening agent	++	++	++	-
Laboratory processing	+	++	+	+
Extrinsic properties of swab shaft (length, thickness, rigidity)	+	++	+	+
Area to fix a traceability tag	++	++	-	+
Easy to seal with security sticker	++	+	-	+

Table A.2 : Practical criteria taken into consideration. The evaluation of these characteristics ranges from - (weakness of the device) to ++ (advantage of the device). The four swabs are in the same price range.

Collars	Comparison	Mean (ng/µl)	Standard deviation	Median (ng/µl)	Wilcoxon p-value
N=20	Cop vs Pri	0.65 vs 0.13	0.98 vs 0.23	0.28 vs 0.05	1.907e-06*
N=20	Pur vs Pri	0.05 vs 0.06	0.06 vs 0.05	0.03 vs 0.04	0.1054
N=20	Sar vs Pri	0.11 vs 0.09	0.11 vs 0.06	0.06 vs 0.08	0.9854

Table A.3 : Comparisons on collars. * Significant test result

Screwdrivers	Comparison	Mean (ng/µl)	Standard deviation	Median (ng/µl)	Wilcoxon p-value
N=20	Cop vs Pri	0.032 vs 0.026	0.045 vs 0.037	0.012 vs 0.014	0.7510
N=20	Pur vs Pri	0.019 vs 0.027	0.031 vs 0.041	0.006 vs 0.012	0.1165
N=20	Sar vs Pri	0.020 vs 0.017	0.023 vs 0.017	0.013 vs 0.012	0.4304

Table A.4 : Comparison on screwdrivers.

Steering wheels	Comparison	Mean (ng/µl)	Standard deviation	Median (ng/µl)	Wilcoxon p-value
N=20	Cop vs Pri	2.82 vs 1.77	10.02 vs 6.74	0.20 vs 0.11	0.0073*
N=20	Pur vs Pri	0.37 vs 0.58	0.59 vs 0.73	0.13 vs 0.24	0.0083*
N=20	Sar vs Pri	1.29 vs 1.39	2.06 vs 2.46	0.61 vs 0.59	0.3118

Table A.5 : Comparison on Steering wheels. * Significant test result