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Performance of Different Cotton and Nylon Swabs on DNA Recovery and Storage

تقييم أداء مسحات القطن والنايلون المختلفة فيما يتعلق باستعادة الحمض النووي وتخزينه

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Abstract

Touch DNA samples are routine yet challenging pieces of evidence that provide investigators with information that helps them solve crimes. However, this type of evidence can be easily lost if the correct collection method is not used. This problem could be overcome with an optimal method of collection that increases the amount of touch DNA collected from different types of surfaces. Better-quality touch DNA can increase the chances of getting a full genetic profile. This study was divided into two parts which aimed to assess whether the type of swab used on different surfaces will significantly increase DNA recovery, concentrations, and the DNA preservation during three different timeframes (24h, 1 month and 3 months). Two different cotton swabs and Nylon swabs were used to lift touch DNA on three different surfaces (glass, plastic and wood) to identify the most suitable method of collection across all three surfaces. A total of 72 samples were lifted (3 replicates from each swab on 3 different surfaces) from two different participants (Male and Female) which were left to dry for 14 days in room tem-

Keywords: Forensic science; DNA preservation; Touch DNA; Trace DNA; DNA collection.

المستخلص

عينات الحمض النووي الناتجة عن التلامس هي أدلة روتينية ولكنها صعبة، حيث تزود المحققين بالمعلومات التي تساعدهم في حل الجرائم. ومع ذلك، يمكن فقدان هذا النوع من الأدلة بسهولة إذا لم يتم استخدام طريقة الرفع الصحيحة. يمكن التغلب على هذه المشكلة من خلال الطريقة المثلى للرفع والتي تزيد من كمية الحمض النووي للعينات الناتجة عن التلامس الذي يتم جمعه من أنواع مختلفة من الأسطح. يمكن لعينة ذات جودة عالية من الحمض النووي الناتج عن التلامس أن تزيد من فرص الحصول على العدد الكامل للسمات الوراثية الجنائية.

قُسّمت هذه الدراسة إلى جزأين يهدفان إلى تقييم ما إذا كان نوع المسحة المستخدمة على الأسطح المختلفة سيزيد بشكل كبير من استعادة الحمض النووي وتركيزات الحمض النووي التي سيتم الحصول عليها وقدرتها على الحفاظ على الحمض النووي خلال ثلاث فترات زمنية مختلفة (24 ساعة وشهر و3 أشهر).

استُخدمت مسحتان مختلفتان من القطن ومسحات النايلون لرفع الحمض النووي الناتج عن التلامس على ثلاثة أسطح مختلفة (الزجاج والبلاستيك والخشب) لتحديد أنسب طريقة لجمع الحمض النووي من الأسطح الثلاثة.

رُفع ما مجموعه 72 عينة (تم تكرار جمع عينات كل مسحة ثلاث مرات على 3 أسطح مختلفة) من مشاركين مختلفين (ذكر وأنثى) وقد تُركت المسحات لتجف لمدة 14 يومًا في درجة حرارة الغرفة قبل استخلاص الحمض النووي منها.

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الكلمات المفتاحية: علوم الأدلة الجنائية، حفظ الحمض النووي، الحمض النووي الناتج عن التلامس، كمية الحمض النووي الضئيلة، جمع الحمض النووي.

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perature prior to DNA extraction. DNA preservation of the swabs was observed while using three dilutions of blood sample which was prepared from one of the volunteers (1:1 – 1:10 – 1:20) where 10 uL of each dilution was pipetted onto the four types of swabs in three replicates (n=36) to observe the preservation over three different timeframes 24h storage, 1 Month and 3 Months with a total of 108 samples. The COPAN CLASSIQSwabs™ Dry swab showed an overall average result during the storage periods of 24h with (1:1) dilution by (2.694ng/µL), (1:10) dilution with (0.548ng/µL) and (1:20) dilution with (0.143ng/µL). Results for the period of 1 Month also showed an average of (1:1) dilution with (2.825ng/µL), (1:10) dilution with (0.361ng/µL) and (1:20) dilution with (0.156ng/µL). These findings can be helpful for laboratories and crime scene investigators to optimize DNA sample collection and preservation based on their workflow.

تم تقييم حفظ المسحات المختلفة للحمض النووي من خلال استخدام ثلاث تخفيفات لعينة الدم التي تم جمعها من أحد المتطوعين، كالتالي: (1: 1 - 1:10 - 1:20) حيث تم وضع 10 ميكرولتر من كل عينة مخففة على الأنواع الأربعة من المسحات، وقد تم تكرار ذلك ثلاث مرات بإجمالي عدد عينات = 36 وذلك لمراقبة قدرة المسحات على الحفظ على مدار ثلاث فترات زمنية مختلفة وهي: التخزين لمدة 24 ساعة، ولمدة شهر واحد ولمدة 3 أشهر بإجمالي عدد عينات يساوي 108 عينة.

أظهرت مسحة COPAN CLASSIQSwabs™ الجافة متوسط نتيجة إجمالية خلال فترات التخزين لمدة 24 ساعة مع تخفيف (1: 1) يساوي (2.694 نانوغرام / ميكرولتر)، وتخفيف (1:10) يساوي (0.548 نانوغرام / ميكرولتر) وتخفيف (1:20) يساوي (0.143 نانوجرام / ميكرولتر). أظهرت نتائج فترة شهر واحد أيضًا مع تخفيف (1: 1) متوسط يساوي (2.825 نانوغرام/ميكرولتر)، ومع تخفيف (1:10) متوسط يساوي (0.361 نانوغرام/ ميكرولتر) ومع تخفيف (1:20) متوسط يساوي (0.156 نانوغرام / ميكرولتر).

يمكن أن تكون هذه النتائج مفيدة للمختبرات والمحققين في مسرح الجريمة لتحسين طريقة جمع عينات الحمض النووي والحفاظ عليها بناءً على إجراءات عملهم.

1. Introduction

It is widely accepted that physical contact leaves a trace, which forensic experts use to identify criminals. DNA evidence is particularly useful in determining whether a suspect was present at the crime scene or had contact with the victim. However, DNA can be easily damaged or lost if proper precautions are not taken [1]. Touch DNA, defined as DNA transferred from skin cells during physical contact, can be recovered using new methods and technologies, and can play a crucial role in identifying individuals linked to a crime. Touch DNA is challenging to analyze in forensic laboratories because the amount of DNA present is typically very low and not visible to the naked eye [2]. Due to the recent advances in DNA techniques and technology, most types of biological evidence can be used to generate a genetic profile, which can be useful in identifying individuals linked to a crime. Unlike blood and other bodily fluids, the cells left at a crime scene through touch are not visible to the naked eye and are typically found in very small amounts [3]. Currently, touch DNA is considered one of the most challenging samples encountered in a forensic laboratory owing to the low quantities of DNA available for analysis, which very often makes it difficult for a full genetic profile to be generated [4]. When an object is touched, only small amounts of epithelial cells are usually left behind, making it difficult to generate a complete genetic profile [5]. Moreover, the amount of DNA found on handled objects can be very small, and there are many variables that can affect the sample, such as the type of surface, the pressure applied, the time between deposition and collection, environmental conditions, collection, and extraction techniques [6]. Additionally, the number of epithelial cells shed by an individual can vary naturally, leading to different amounts of transferred cells in different circumstances. DNA transfer can occur through direct contact between an individual and a surface or object, or indirect when the DNA of multiple individuals on a surface or object is transferred through subsequent touch [7]. This study has investigated the optimal swab type to use on the most encountered

surfaces by forensic investigators and to asses the persistence of DNA during different periods of time which can be helpful for forensic laboratories to understand how time can affect DNA quality and use appropriate storage conditions for better results for casework samples.

2. Materials and Methods

2. 1 Selection of Swabs

Currently, the cotton swabs TubSWAB Transport Swab by PorLab (PorLab Scientific Co, China) (C1) is used for routine DNA collection of evidence found in crime scenes. Alternatively, another cotton swab by COPAN CLASSIQSwabs Dry Swabs (159C) (C2) is considered for the study to evaluate and compare the DNA collection between them. Nylon swabs were also introduced in this study to evaluate between the two types swabs and identify which yields more DNA [8], [9]. The nylon swabs selected were COPAN 4N6FLOQSwabs™ (Copan Italia S.p.A., Italy) (N1) and the Puritan HydraFlock (Puritan Medical Products, USA) (N2).

2. 2 Substrates

Substrates play a huge role in influencing the amounts of DNA recovered to generate a DNA profile from Touch DNA which enables police units to identify crime offenders [10] [11]. Accordingly, for the first part of our comparative study, three substrates were selected for their well-known appearance in most crime scenes investigations and used to lift DNA by forensic unit (Glass, smooth non-porous; Wood, rough porous and textured plastic, rough non-porous). All non-porous surfaces were cleaned with 2% virkon (viricidal disinfectant) and ultraviolet radiation (UV) for 15 min; porous surfaces were irradiated using UV light for 20 min prior to the DNA deposition.

2.3 First Part: DNA sampling and collection

Two individuals were instructed to wash their hands with antibacterial soap and refrain from any activity for five minutes. They were then asked to touch behind their ears or forehead to transfer eccrine sweat and load their fingers with DNA. Using their index, middle, and ring fingers separately, they were asked to touch surfaces and apply medium pressure for one minute on a 25 x 75 mm area of the surface. They repeated this process three times on three different surfaces, waiting 30 minutes between each repeat, before returning to their normal office work. The procedure was repeated for a total of 72 samples (three replicates for each swab). Before collecting the samples, a 7cm spray of sterile distilled water was used to moisten the swab using a plastic spray bottle (each single spray contains approximately 50 µL). The Touch DNA was collected and extracted at room temperature 14 days after deposition.

2.4 Second part: DNA preservation on swabs

Following the protocols validated in the lab, samples are routinely stored at RT before being analyzed. Although RT storage is appropriate since it does not require cooling devices, studies have shown that DNA damage may already occur a few hours after collection in climates which show a very strong degree of heat such as The Middle East during summer [12]. It is vital to understand how temperature and storage duration can affect DNA preservation on swabs since forensic laboratories around the world differ in terms of storage periods and climates, thus certain protocols can damage DNA samples [13]. In this part, we considered blood samples over Touch DNA taking into consideration that blood is a strong sample for DNA analysis over Touch DNA which can be affected by many factors [7]. Therefore, three

dilutions of blood sample were prepared from one of the volunteers (1:1 – 1:10 – 1:20) where 10 uL of each dilution was pipetted onto the four types of swabs in three replicates (n=36) to observe the preservation over three different timeframes 24h storage, 1 Month and 3 Months with a total of 108 samples.

2.5 DNA extraction and quantification

COPAN swab heads were broken off at the breaking point, while the TubSWAB Transport Swab by PorLab (PorLab Scientific Co, China) (C1) and Puritan HydraFlock (Puritan Medical Products, USA) (N2) were cut with sterile scissors just under the head. DNA extraction was performed with Cartridges from PrepFiler™ Express forensic DNA extraction kits (4441352; Applied Biosystems) which were run on an Automate Express forensic DNA extraction system (4441763; Applied Biosystems). Samples were lysed using the lysis buffer provided with the kit supplemented with 10 mmol/L dl-dithiothreitol (43815; Sigma-Aldrich) for 40 minutes at 70°C. Approximately 50 µL elution volume was generated from each sample by Automate Express System.To assess DNA yields, Investigator® Quantiplex Pro Kit DNA Quantification Kit (387216; QIAGEN) was performed according to the manufacturer's recommendations using Real-Time PCR 7500 system (4351105; Thermo Fisher Scientific).

3. Results

3. 1 First Part: DNA sampling and collection

Typically, Touch DNA that is on a surface can be limited to various factors, and one of those factors are shedder status of a person [14]. The amount of DNA a single person leaves on a particular surface differs from one person to another. Volunteers in this trail showed that the overall Touch DNA amounts on same measured circumstances were different. Looking at quantities of DNA lifted from Glass of both volunteers we can clearly see a major difference in terms of DNA average collected (Figure 1) with C2 swab the most DNA yield (0.0321ng/µL) and N1 with the least amount of DNA yield (0.0032ng/µL). However, DNA quantities collected from Wood from both volunteers differed, with volunteer 1 having C2 swab as the most DNA yield (0.0067ng/µL) and C1 as the least (0.0018ng/µL), volunteer 2 DNA results showed that C1 DNA yield is highest (0.0016ng/µL) and N1 as the least (0.0004ng/µL). Quantities collected from Plastic had the highest DNA yield with shared swab results between volunteers with C2 as the highest (0.1120ng/µL) and N2 with the least amount of DNA recovered (0.0002ng/µL).

3. 2 Second part: DNA preservation on swabs

The quality of DNA from the blood dilution sets deposited on the four different swabs did not show a significant difference between the 24h and 1 Month samples which remained quite stable (Figure 2) with C2 swab the highest between the swabs in terms of DNA preservation in RT condition. However, there is a drop in DNA quantities in swabs preserved for 3 Months over the three sets of dilution with N2 swab the most DNA quantity available in the (1:1) dilution with (2.945ng/µL) and N1 for the (1:10) with (1.170ng/µL). The notable result was for the (1:20) dilution with C2 with the highest DNA quantity (0.876ng/µL).

4. Discussion

Since many factors come along to denote the quality and quantity of Touch DNA, it is important for all forensic laboratories to understand the nature of such samples since nearly most laboratories around the world have variable difficulty in DNA recovery

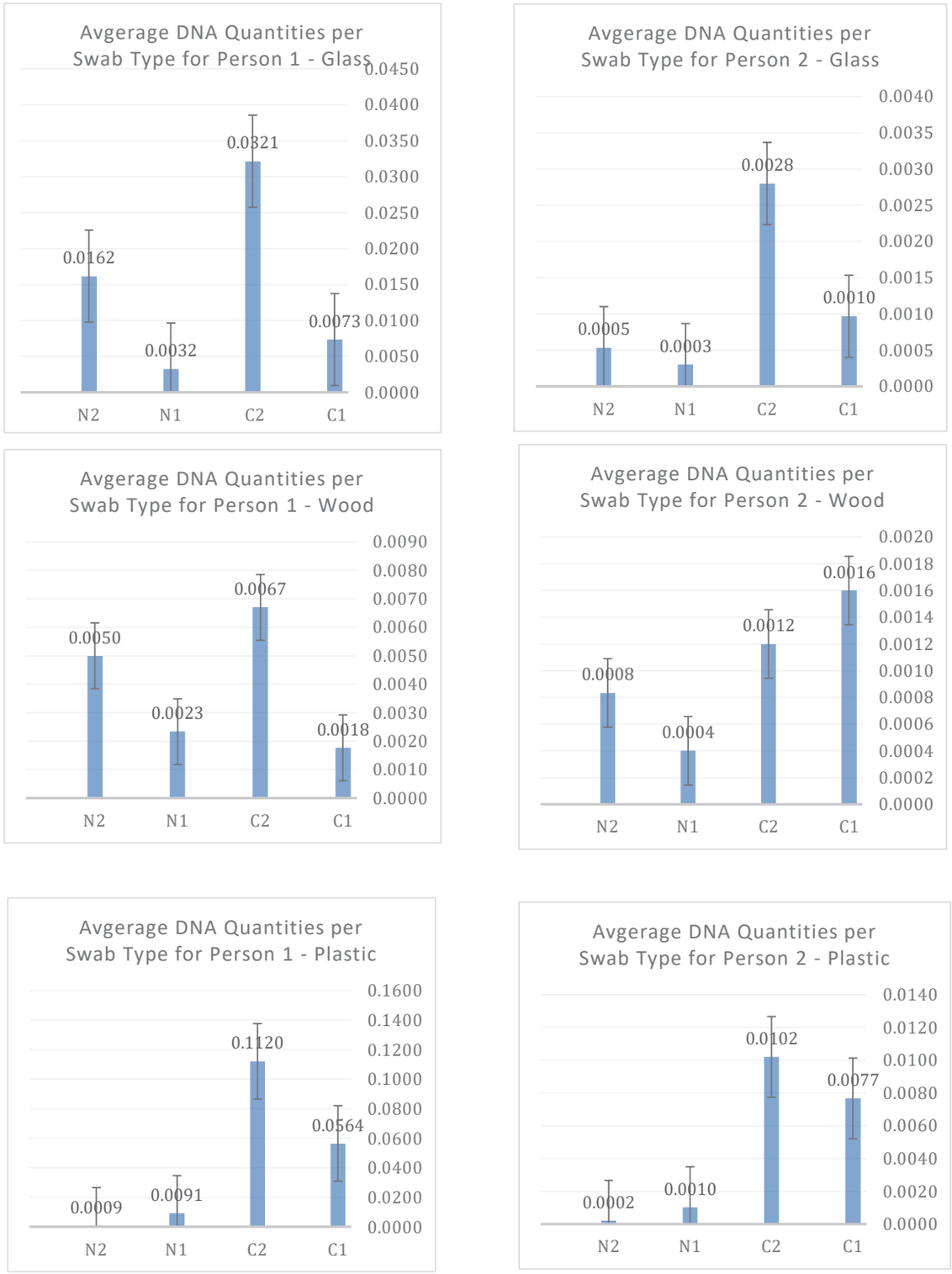

Figure 1- *Average Touch DNA quantities collected from three surfaces, Glass (n=24), Wood (n=24) and Plastic (n=24) with three replicates of each swab with a total of 72 samples. (Quantities shown above are all in (ng/µL))*

from crime scene samples. Touch DNA can play a vital role in helping solve crime, however the methods used to recover the samples should be validated to ensure higher DNA amounts being lifted. Over the years, many studies showed that the cotton swabs would trap the biological material and not release enough DNA which would be enough to generate a genetic profile for comparison [8], [15], [16]. In our study, we focused on two different swab type (Cotton and Nylon) to understand the best swab to recover and preserve DNA. During the first part of the study, we used three different surfaces (Glass, smooth non-porous; Wood, rough porous and textured plastic, rough non-porous) to test the recovery of DNA. Results (Figure 1) show that the cotton swabs show better overall performance especially the cotton COPAN CLASSIQSwabs Dry Swabs (159C). This particular swab performed well across all the surfaces with one exception for the wood surface for volunteer 2. Moreover, the data also shows that the Puritan HydraFlock (Puritan Medical Products, USA) has a better DNA yield than the COPAN 4N6FLO-

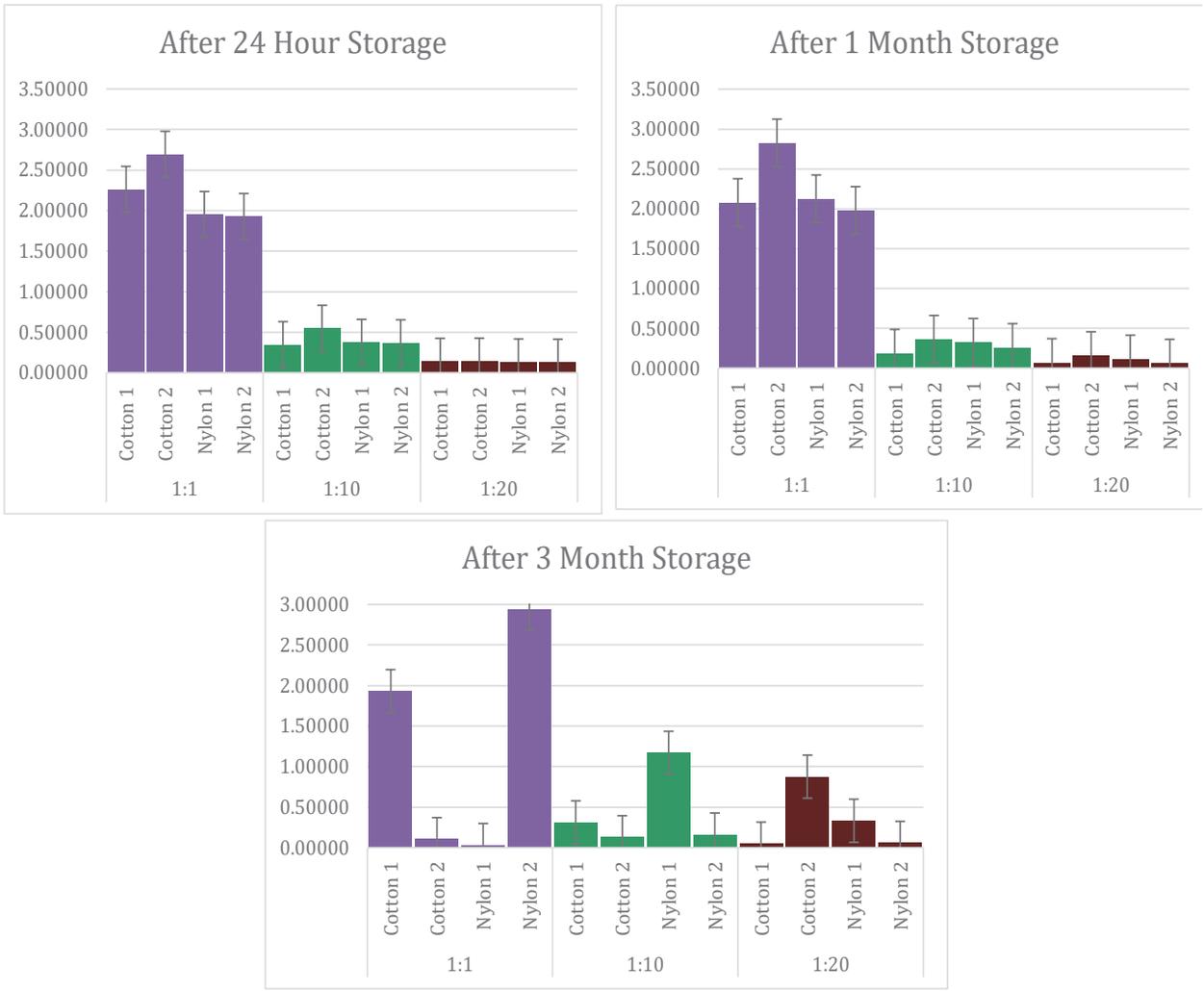

Figure 2 - *Average Blood DNA preservation quantities collected from the four swabs with three different dilutions, 1:1 (n=36), 1:10 (n=36) and 1:20 (n=36) with three replicates of each swab with a total of 108 samples. (Quantities shown above are all in (ng/µL))*

QSwabs™ (Copan Italia S.p.A., Italy). In the second part of the study, the COPAN CLASSIQSwabs Dry Swabs (159C) appear to have to best ability to release DNA from the swabs compared to the others. Through the DNA preservation part of the study, based on many studies [13], [17], [18] we left the packaging open until the swab was dry for the duration period at RT. Results showed no difference between 24h and 1 month in storage and thus not much of a drop in the DNA quantity when stored up to one month. However, the major difference in quantity was in the 3 months' period. A possible cause of the DNA yield difference in the 3 months period is the development of fungal growth on the swab heads, which can be a possible cause to the major difference in the results since the swabs were left to dry in RT with humidity as factors in the development of enzymes of the microorganisms [19]. Is it difficult to point that the cotton swab is a better option for storage than the nylon since every forensic laboratory use an alternative DNA extraction and quantification kits for their casework samples. Nevertheless, Results differ based on factors such as volunteer size and the shedder status. People tend to shed DNA differently based on their daily routine and activities. Another limitation for the study is the number of samples being tested for the study which can add more qualitive results for the analysis. Taking everything into account, forensic experts will not be able to depend on one collection method without validating the results and looking at the available literature to get a clear understanding that different swabs can be utilized for various surfaces and substrates. Findings of the study suggest the freezing of swabs which will improve the DNA availability in terms of long-term storage and avoid degradation. Immediate extraction of the samples is suggested to avoid DNA loss which was shown over a period of 14-day RT storage. Moving forward with STR profiling for future research can also give forensic DNA experts a better understanding in terms alleles being present which in return can determine the best optimal approach for their casework samples.

5. Conclusion

It is vital for all DNA forensic laboratories to always achieve the best overall results during DNA profiling casework which will enable police forces to put offenders behind bars. Based on our results, the cotton COPAN CLASSIQSwabs Dry Swabs (159C) (C2) had a better overall performance in terms of collection with DNA yield (0.0321ng/µL) in Glass and (0.1120ng/µL). However, in terms of preservation the (C2) swab also had the highest DNA yield storage periods for 24h with (1:1) dilution by (2.694ng/µL), (1:10) dilution with (0.548ng/µL) and (1:20) dilution with (0.143ng/µL). Results for the period of 1 Month also showed an average of (1:1) dilution with (2.825ng/µL), (1:10) dilution with (0.361ng/µL) and (1:20) dilution with (0.156ng/µL) Nevertheless, forensic laboratories should always consider the degradation of swabs during a period of time if left at RT. We advise the immediate extraction of Touch DNA samples for the low quantities available prior of extraction. We also suggest the freezing of swabs for long term storage if the logistics were available to prevent the degradation of the samples. We believe that the results should encourage forensic DNA analysts to consider such factors when looking to validate new methods for their daily workflow.

Conflict of interest

The authors declare no conflicts of interest.

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Authors' contributions

Mohammed Alrahma: DNA collection , writeup of the paper; Hanan Almulla: DNA collection , writeup of the paper; Suaad Alshehhi: DNA Extraction and quantification; Maryam Almuhairi: DNA Extraction and quantification; Naima Aljanahi: DNA Extraction and quantification; Ayesha Alsabhan: DNA Collection; Hussain Alghanim: Overall supervision and DNA analysis.

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