STR profiling of DNA obtained from improperly stored cotton swabs

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Abstract

Improper packaging and storage of cotton swabs containing biological sample is commonly known to lead to fungal growth, which results in the degradation of valuable DNA evidence. Nonetheless, there are certain cases where DNA profiles must be generated from these contaminated swabs, due to their rarity or lack of opportunity to re-collect the samples. As a result, this study aimed to identify the likelihood of obtaining useful DNA profiles from the fungal contaminated cotton swabs. Wet cotton swabs used to collect dried saliva stain, were packaged immediately in plastic bag and stored at room temperature to simulate improper condition. STR profiles were generated using AmpFlSTR® Identifiler™ PCR Amplification kit. Results showed that useful DNA profiles could still be generated from cotton swabs stored at room temperature up to 2 days of storage time (Power of discrimination = 9.57×10^{-17}).

Keywords: STR profiling, cotton swabs, degraded DNA

1. Introduction

DNA analysis is a powerful investigative tool in forensic science. Biological evidence such as blood, saliva, semen, and skin cells contain DNA, which are valuable for human identification, crime scene reconstruction, and kinship analysis. For this reason, it is necessary to collect, handle, and store DNA evidence properly by following guidelines to decrease the chance of cross contamination and DNA degradation, as well as ensuring that the evidence can be legally accepted in court.

One of the most common methods for collecting biological evidence is swabbing technique. The FBI laboratory guideline states that swabs should be air-dried before packaging in suitable containers, such as cardboard boxes, for ventilation (FBI Laboratory Publication Federal Bureau of Investigation, 2013). DNA evidence needs to be stored and maintained in cool and dry conditions to prevent bacterial and fungal growth (Baust, 2008), since microorganisms cause random cleavage of DNA by nuclease enzymes. In addition, they generate free radicals that lead to DNA damage (Alaeddini *et al.*, 2008). However, due to inconvenience or negligence, improper collection and handling of the DNA evidence, for example, immediate packaging of wet swabs, the use of wrong container, or storage/transportation of evidence in poor conditions can still occur. Since the collected DNA evidence cannot be reproduced, the DNA analysts may still need to process the valuable evidence, even though its quality has been compromised from microbial proliferation.

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In this study, the maximum duration that improperly stored swabs containing biological evidence can still yield useful DNA profiles was investigated. The observation of fungal growth was used to indicate the state of the microbial contamination of the evidence, as it is difficult to detect the growth of bacteria by naked eyes. Moist cotton swabs which had been used to collect saliva stain were kept in sealable plastic bags at room temperature for 1, 2, 5, and 30 days. STR profiling was performed on DNA extracted from these swabs, and the profile quality was then assessed. The results can be used to help DNA analysts in deciding whether it is worth processing such compromised biological evidence further.

2. Methods

2.1 Sample preparation

All experiments were performed in triplicates. Dried saliva stains were prepared by collecting 2 ml of saliva, and aliquots of 100 µl were dropped on to petri dished and allowed to dry at room temperature overnight (total number of samples = 15). One hundred microliters of sterile deionized water was used to moisten sterile cotton swabs. Swabbing were carried out in one direction while rotating the stick twenty times then changed to the opposite direction.

To simulate improper packaging and storage conditions, the cotton swabs were immediately packaged in sealable plastic bags and stored at room temperature for 0, 1, 2, 5, and 30 days. The 0-day were used as positive control for sample preparation. The swabs were observed by naked eyes for fungal growth. Buccal swabs were prepared by using sterile cotton swabs to rub the inner side of the cheek for 15 times. The DNA was extracted from these swabs and used as positive control in the CSF amplification and STR profiling.

2.2 DNA extraction

Cotton swabs were extracted by Wizard® SV Genomic DNA Purification (Promega Corporation, USA) following the manufacturer's protocol (Promega Corporation, 2012).

2.3 DNA quantification

NanoDrop™ 1000 Spectrophotometer (Thermofisher Scientific, USA) and Qubit® 3.0 Fluorometer (Thermofisher Scientific, USA) were used for DNA quantification following the manufacturer's protocol for genomic DNA quantification (Thermofisher Scientific, 2008, 2014).

2.4 CSF amplification

In order to assess the degree of DNA degradation, the DNA samples were amplified using primers (CSF-F and CSF-R; Roumruk, 2014 and Swango *et al.*, 2006, respectively) for an intron region of the human *c-fms* proto-oncogene for the CSF-1 receptor gene (Swango *et al.*, 2006), which is upstream to the CSF1PO repetitive sequence. The expected amplicon size of CSF primers is 156 bp. PCR was performed using 50 ng of DNA template. Thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at

60°C for 30 sec, extension at 72°C for 1 min, and then final extension at 72°C for 7 min. PCR products were separated on 2% (w/v) agarose/TBE gel containing ethidium-bromide. Electrophoresis was performed at 70 volts for 90 min. The DNA fragments were visualized using G-Box Gel Documentation (Synoptics Ltd, UK).

2.5 DNA profiling

Two nanograms of extracted DNA were amplified using AmpFlSTR® Identifiler™ PCR Amplification kit (Applied Biosystems, USA) for 28 cycles, and STR fragments were separated by capillary electrophoresis (ABI Prism® 310 Genetic Analyzer, Applied Biosystems, Foster city, CA). Peak detection was set from 75 to 450 bases. The stochastic and analytical thresholds of 50 RFU were applied in peaks and alleles calling. Results were analyzed using GeneMapper Software (Applied Biosystems, USA).

3. Results and discussion

3.1 Visual examination of swabs

Examination by naked eyes of the cotton swabs, which had been packaged immediately without being air-dried in sealable plastic bags at room temperature, showed that fungal growth could be detected on 1 in 3 swabs which had been stored for 5 days and 2 in 3 swabs which had been stored for 30 days (Fig.1).

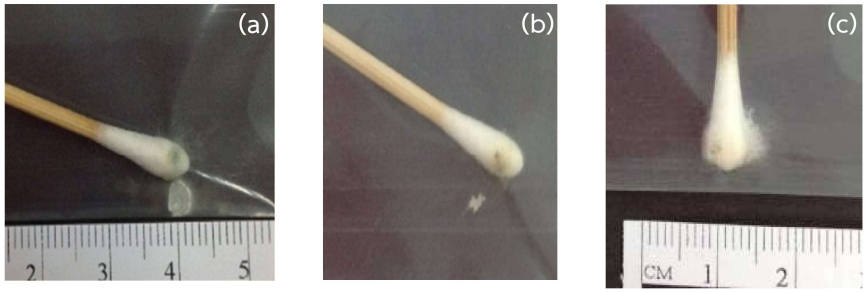

Fig. 1. Fungal growth on moistened cotton swabs. Fig. 1a Fungal contaminated swab from replicate No.1 at day 5. Figs. 1b and 1c are fungal contaminated swabs from replicate No. 1 and No. 2 at day 30.

3.2 Effects of storage time on DNA quantity and quality from swabs stored in improper conditions

The quantity of DNA in the samples was estimated using NanoDrop™ 1000 Spectrophotometer and Qubit® 3.0 Fluorometer, and the results are shown in Fig. 2. In general, it could be seen that the DNA concentration from both measurements decreased as the storage time increased when compared to day 0. The reduction in amount of DNA indicated that DNA continued to be degraded from day 0 of storage time. While data from Qubit® 3.0 Fluorometer showed that the DNA concentration continued

to decrease as the storage time increased, the NanoDrop™ 1000 Spectrophotometer results did not show the similar trend (Fig. 2).

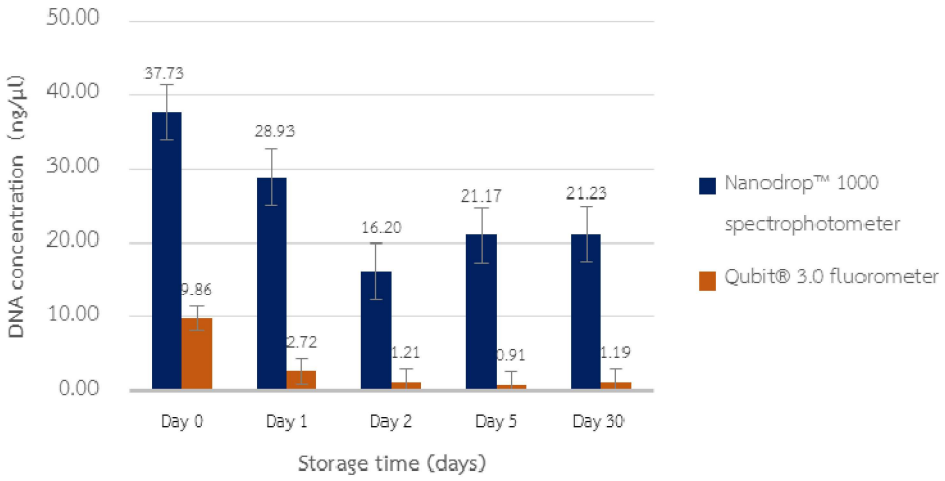

Fig. 2. The average DNA concentration obtained from 3 replicates of saliva sample-containing cotton swabs which had been stored wet in plastic bags at room temperature.

This could be due to the fact that NanoDrop™ 1000 Spectrophotometer measures the DNA quantity based on its absorbance at 260 nm. However, this is not specific only to DNA, since RNA also absorbs at this wavelength. Also, although their maximum absorbance wavelength is not 260 nm, high concentration of aromatic amino acids in the samples may interfere with the absorbance at 260 nm (Schmid, 2001). As a result, the DNA quantity in the sample could be overestimated when quantification is performed using UV spectrophotometry. In order to improve specificity, the Qubit® 3.0 Fluorometer was used to measure the DNA quantity. The DNA quantification by Qubit® 3.0 Fluorometer employs a dye that emits fluorescent signal only when it is bound to DNA. This means that the signal being used for quantity calculations are generated specifically from DNA that is present in the sample, and therefore the method offers more reliable measurements of the DNA quantity. The ratio of absorbance at 260 nm to absorbance at 280 nm, which could only be obtained from the NanoDrop™, was used to assess the purity of DNA.

After PCR was performed and the products were separated by gel electrophoresis, the results showed that the intensity of the DNA bands decreased as storage time increased (Fig.3). This is in accordance with the results of DNA quantity measurements using Qubit®3.0 Fluorometer. It could be seen that the intensity of the DNA bands at 5 and 30 storage days were very low in contrast to the DNA quantity measured. This may be due to the fact that DNA from microbes interfered with the estimation of true amount of DNA from dried saliva sample on these cotton swabs.

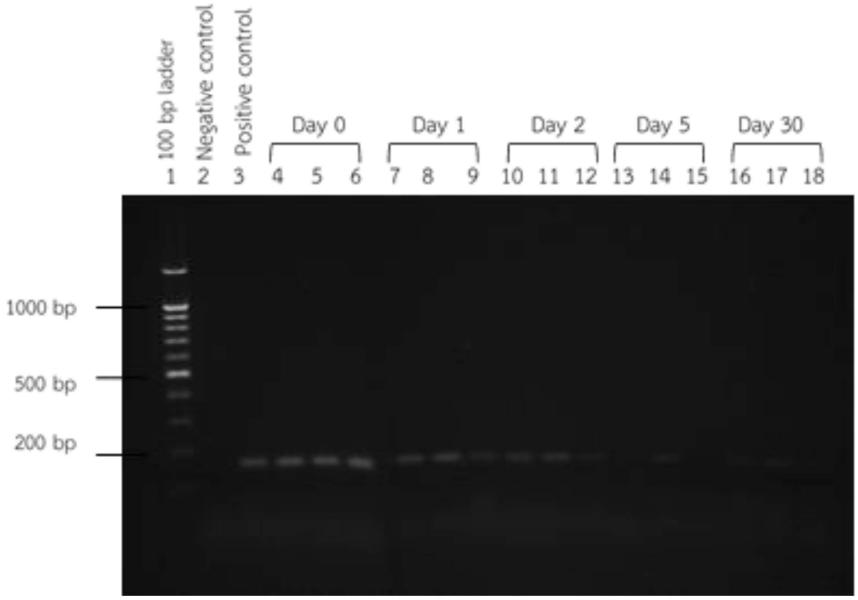

Fig. 3. PCR products from amplification of 50 ng of DNA from cotton swabs moistened with sterile deionized water at 0, 1, 2, 5 and 30 days. The gel electrophoresis was performed using 2% (w/v) agarose/TBE gel containing ethidium bromide.

3.3 STR profiling of DNA obtained from swabs stored in improper conditions

Table 1 showed the results from STR profiling. DNA samples from 0 and 1 day of storage generated full DNA profiles, while DNA samples stored for 2, 5 and 30 days generated incomplete DNA profiles. Fungal contaminated swabs at 5 days of storage time could also generate DNA profiles up to 8 STR loci with the overall profile frequency of 1.47×10^{-11} or about 1 in 68 billion, which is more than a million times the population of Thailand (Worldometers, 2016). Only 2 STR loci were generated from the DNA samples from 30 days of storage time, and the profile frequency was 4.99×10^{-4} or about 1 in 2000, and therefore cannot be used to identify an individual. It should be noted that sex could be typed for all samples. Based on these results, useful DNA profiles could be obtained from swabs stored in these conditions for up to 2 days, which is when there was no fungal contamination observed.

Products from the metabolism of microbes are one of the factors that cause DNA degradation. The microorganisms generate free radicals such as hydroxyl radicals through the metabolic activities of aerobic bacteria (Alaeddini *et al.*, 2008). Free radicals lead to non-enzymatic DNA degradation process by oxidative reaction. The oxidative damage due to free radicals converts cytosine and thymine into hydrations, resulting in inhibition of PCR amplification (Alaeddini *et al.*, 2008).

Table 1 Results of the STR profiling from swabs stored at room temperature for 0, 1, 2, 5 and 30 days. The median of the number of allelic peaks and the number of STR loci generated from 3 replicates.

	Median No. of allelic peaks (complete profile = 30)[1]	Median No. STR loci (complete profile = 15)[2]	Profile frequency[3] (Random Match Probability)
Positive control	30	15	1.94×10^{-22} (1 in 5.15 Sextillion)
Day 0	30	15	1.94×10^{-22} (1 in 5.15 Sextillion)
Day 1	30	15	1.94×10^{-22} (1 in 5.15 Sextillion)
Day 2	22	11	9.57×10^{-17} (1 in 0.01 Quintillion)
Day 5	13	8	1.47×10^{-11} (1 in 68 Billion)
Day 30	4	2	4.99×10^{-4} (1 in 2,000)

[1] Not including Amelogenin peaks which were present in all profiles.

[2] 15 STR loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA).

[3] Rerkamnuaychoke *et al*. (2006)

4. Conclusion

The effects of improperly storing cotton swabs which had been used to collect forensic biological evidence have been demonstrated in this study. The DNA quantity and quality on cotton swabs that were packaged in sealable plastic bags while still wet and stored at room temperature decreased as the storage time increased, especially after 5 days, when fungal growth could be observed on the swabs. However, the results seemed to suggest that even though swabs had been left in non-optimal conditions it may still be possible to obtain useful DNA profiles (>10 loci detected), if fungal growth could not yet be observed.

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