

## DNA RETRIEVAL ON FLIP-FLOPS

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### Abstract

Flip-flops, a kind of footwear that keeps submitting to Central Scientific Crime Detection Division, Royal Thai Police, for the analysis of contact or trace DNA. In practice there was little success in generating complete DNA profiles. Currently there is limited research on DNA analysis of footwear. Moreover, no paper has ever been published or studied on flip-flops.

This paper aimed to investigate the presence of trace DNA and DNA inhibitor from pre-wear flip-flops. We also examined the post-wear flip-flops and compared post-wear DNA against time spending under normal use. The results indicated no PCR inhibitor present on the pre-wear flip-flops, and only two of the twenty pieces of flip-flops revealed minute amount of DNA on pre-wear samples. DNA quantity could be estimated from post-wear samples with the ascending yield over time. Unexpectedly, the decline in DNA quantity occurred in 8 of the 20 samples at the 14-day experiment. The decline is more likely due to the microbial decomposition process. The average DNAs on the post-wear flip-flops from 1, 7, and 14-day experiments were 0.016827, 0.410868, and 0.443817 ng/μl respectively. Further results indicated that 95% Et-OH swab could recover greater DNA yields compared to sterile distilled water swab. This current study indicates that 95% Et-OH solution is suitable to use with the double swab technique for trace DNA recovery in sample that does not need extra time in swabbing and the quantity of trace DNA from worn flip-flops can be estimated.

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Keywords: Flip-flops; Trace DNA

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### Introduction

Every single piece of evidence recovered in a crime scene has evidential value in terms of forensic aspect. The association of victim, scene and perpetrator must be revealed for a reconstruction of the crime and the resultant DNA is often a crucial tool to identify a perpetrator or exclude an innocent suspect. Central Scientific Crime Detection Division (CSCDD), Royal Thai Police, is one of the forensic science service authorities that have provided forensic services for society. DNA analysis is one of those services that have been served for over the past 8 years. In practice, CSCDD has successfully produced complete DNA profiles from most evidence submitted but has little success in flip-flops; a kind of footwear frequently found or inadvertently left behind at a crime scene. The absence of other evidence in a crime scene, the resultant profile obtained from this footwear will definitely be of evidential value. Flip-flops are regarded as trace evidence. The quantity and quality of DNA recovered from such evidence depends on a wide range of factors. Therefore forensic DNA scientists need to reconsider the basic properties of trace evidence including the transfer process, the persistence and the abundance of trace DNA<sup>(1-2)</sup>. The tendency to shed skin cells onto handled objects very varies among individuals and the factors affecting the transfer process as well as the shedding ability were discussed in the past research<sup>(3-10)</sup>. Interestingly, the top of the foot gave greater DNA yields than the sole while the insole area of a suitable shoe found to give adequate DNA to generate profiles<sup>(11)</sup>. Dealing with trace evidence needs efficient sample collection methods. Therefore, several publications of improving DNA recovery were reported and the double swab technique were recommended<sup>(12-13)</sup>. Moreover, bacterial DNA often interferes in the specificity during the quantification but had no effect on the Quantifiler™ calculated DNA concentrations which is specific to human DNA and gave reliable results<sup>(14-15)</sup>. This paper aimed to investigate the presence of trace DNA from pre- and post-wear flip-flops and compare post-wear DNA against time spending under normal use.



#### Materials and methods

Volunteers' feet were first examined for DNA shedding ability by double swab technique using 95% ethanol (Et-OH) dampened cotton swab at the right foot while using sterile distilled water (D.W.) at the left one. DNA was extracted separately from each swab by using the Applied Biosystems DNA QIAamp Microkit and quantified using Quantifiler™ Human DNA Quantification kit. All pre-wear flip-flops were preliminarily screened for pre-existing DNA by using the same process as described previously. At the quantification step, a known DNA concentration was used as a DNA positive control and monitored for PCR inhibitor. Later, all flip-flops were thoroughly cleaned by wiping off with 95% Et-OH dampened gauze repeated two times consecutively. Volunteers were then asked to wear the processed flip-flops at three intervals; 1, 7, and 14 days. For each pair of flip-flops, the right piece of was swabbed by using 95% Et-OH while the left using D.W. DNA extraction and quantification were performed as described previously. Total volume of DNA solution per tube was 50 µl.

#### Results

Of the ten pairs of flip-flops, only two pieces of flip-flops revealed minute amount of 0.0043 and 0.0233 ng of human DNA and no PCR inhibitor present on the pre-wear samples. (Data not shown). To determine each individual shedding ability, DNA quantity retrieved from volunteers' feet swabs was done and ranged from 0.00341 to 0.07381 ng/µl (table 1). The 95% Et-OH swab gave higher DNA yield than D.W. The DNA quantity increased over time for both swabbing solutions in most samples. Unexpectedly, the decline in DNA quantity occurred in 8 of the 20 samples at the 14-day experiment (Table 2 and Figure 1). This unusual event is more likely due to the microbial decomposition process. Further results indicated that in 60% of the samples, 95% Et-OH swab could recover greater DNA yields than sterile distilled water swab.

#### Discussion and conclusion

A past research applied the double swab technique to the hands and feet of sex volunteers. Swabbing solution was not identified and used different methods of extraction and quantification and reported that lower DNA amounts were obtained from the feet compared to the hands.<sup>(11)</sup> In this study we focused on volunteers' feet only. DNA retrieved from volunteers' feet through the double swab technique was shown in table 1. The hypothesis of the study was that the more time volunteers spending on flip-flops the more DNA concentration should be recovered. The results showed that only 55% of the 1-day experiment samples could successfully recover DNA whereas no DNA revealed in the rest 45%. The concentration recovered was zero to very low. This could be due to insufficient physical contact and the variation among individuals in skin cell shedding<sup>(1,5,7)</sup>.

	Et-OH	D.W.	Total
Male1	0	0.00341	0.00341
Male2	0.05072	0	0.05072
Male3	0.05222	0.00455	0.05677
Male4	0.01394	0	0.01394
Male5	0.06528	0.00853	0.07381
Average	0.03643	0.0033	0.03973
Female1	0.01885	0	0.01885
Female2	0.0181	0	0.0181
Female3	0.01885	0.00465	0.0235
Female4	0.02203	0	0.02203
Female5	0.01395	0	0.01395
Average	0.018356	0.00093	0.019286

Table 1. DNA recovered from volunteers' feet using cotton swabbing (ng/μl)

Volunteers		Et-OH			D.W.			Total
	1 day	7 days	14 days	Et-OH	1 day	7 days	14 days	D.W.
Male1	0	0	0	0	0	0.02205	0	0.02205
Male2	0	0.21417	0	0.21417	0.01461	0.08796	0.09795	0.20052
Male3	0.00392	0.11149	0.04217	0.15758	0	0	0.05067	0.05067
Male4	0.03906	0.209	0.3602	0.60826	0.01232	0.04053	0.0317	0.08455
Male5	0	0.15514	0.17109	0.32623	0.02101	0.00806	0.06863	0.0977
Average	0.008596	0.13796	0.114692	0.261248	0.009588	0.03172	0.04979	0.091098
Female1	0.00414	0.02017	0	0.02431	0.00833	0	0.02921	0.03754
Female2	0.0132	0.03676	0.09229	0.14225	0	0.03747	0.03376	0.07123
Female3	0.00364	0.05334	0.094262	0.151242	0	0.16899	0.138914	0.307904
Female4	0.03741	0.0584	0	0.09581	0	0.05309	0.05172	0.10481
Female5	0.01063	0.13216	0.07036	0.21315	0	0.03637	0.08228	0.11865
Average	0.013804	0.060166	0.051382	0.125352	0.001666	0.059184	0.067177	0.128027

Table 2. DNA retrieved from post-wear experiment (ng/μl)



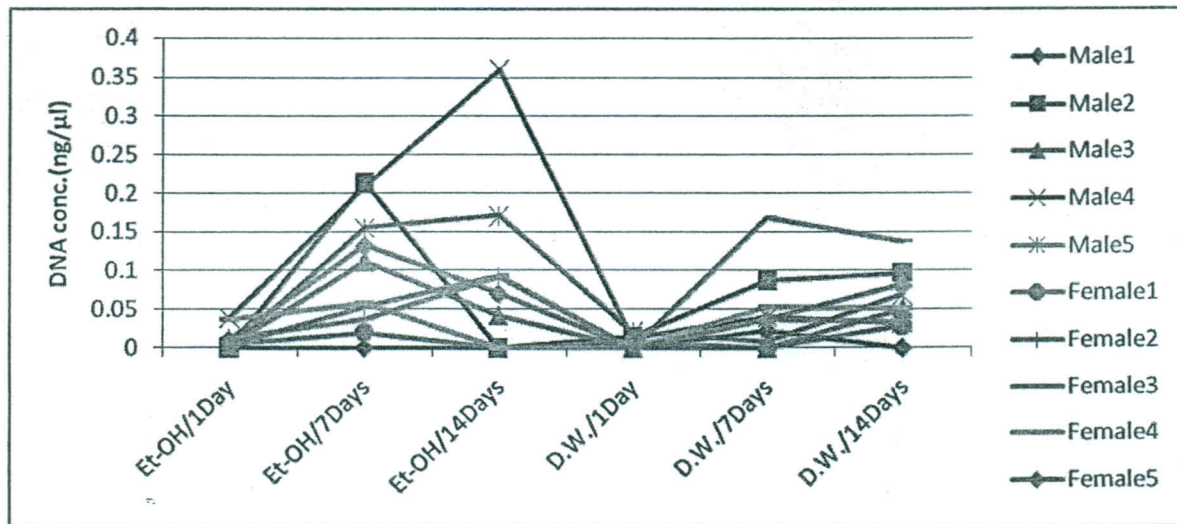


Figure 1. Total DNA quantities recovered from 1-Day, 7-Day and 14-Day experiment by 95%Et-OH and D.W. swab

As expected, the increasing of DNA amount could generally be observed in 90% of samples at 7-day experiment compared to 1-day experiment. But 40% of samples from 14-day experiment unexpectedly showed sign of declined DNA amount compared to the 7-day. This indicated that time length was not the only major affect on DNA quantity. The decline is more likely due to the process of microbial decomposition<sup>(11)</sup> as dirt could obviously be seen during the swabbing of the 14-day experimental samples. Actually there is combination of factors involved. The variability of individuals in cell shedding ability<sup>(1,5,7)</sup> was the very first factor to be considered. The previous researchers also studied on shoe insoles from 11 shoes collected from two donor wearers and reported that the insole area gave adequate DNA to produce profiles. For a shoe, the area in contact with the top of the foot may be the suitable area for sampling<sup>(11)</sup>. In this study the swabs were applied on the entire area of flip-flops in contact with the sole and the top of the foot. Additionally, the solution used in the collecting can also affect the outcome. When comparing the two solutions used in this study, the results showed that 95% Et-OH swab recovered total DNA yields almost two times higher than D.W. (Fig.1) The only disadvantage of 95% Et-OH is that the rapid evaporation property of ethanol. Therefore it may not be suitable for large area or sample that needs extra time of swabbing. The swab could turn dry before finishing. Once it turns dry, the efficiency drops down. This occurrence could also be the cause of the decline of DNA quantity. As more time was used when swabbing the dirt area of the samples. The solution for this issue is the multiple swab technique. When the first swab turns dry, two or more swabs with the solution should be applied. The previous paper also indicated that increasing the number of swab would increase DNA yield<sup>(11)</sup>

For conclusion, the current study indicates that 95% Et-OH solution is suitable to use with the double swab technique for trace DNA recovery in sample that does not need extra time in swabbing and the quantity of trace DNA from worn flip-flops can be estimated. An additional study of 30-day experiment is undertaken to further investigate the effect of time and the test of DNA persistence is also underway.

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