DNA recovered from biological evidence by water-soluble tape lifting

Phattranit Thanawirojn[1], Achirapa Bandhaya[1] and Nathinee Panvisavas[2,*]

Abstract

Cotton swab or swabbing technique has been applied in routine bio-evidence collection. However, evidential material loss occurs during the swab extraction step. This may be critical when small amount of biological trace was present. Therefore, an alternative evidence collection method using water-soluble tape-lifts was investigated. The use of water-soluble tape-lifts was compared to the routine swabbing method on biological sample and biological trace sample that were prepared from saliva and fingerprint. Then DNA was extracted by 3 different methods; Chelex method, modified rapid alkaline lysis method, and DNA IQ™ extraction kit. Results demonstrated that the water-soluble tape can be used as an alternative evidence collection method to collect the biological evidence. There was no inhibitory effect on PCR when using the water-soluble tape as a collection device regardless of the extraction methods tested. DNA yield recovered depended on the nature of evidence and DNA extraction method.

Keywords: DNA recovered, water-soluble tape, biological evidence

1. Introduction

In forensic investigation, DNA is one of the most important evidence and is routinely used to identify the individuals. DNA can be recovered from biological evidence such as biological fluids and their stain as well as biological trace evidence such as fingerprint, lip print**.** The standard method for biological evidence collection is cotton swab technique. However, small amounts of biological materials retained on the swab, thus causing loss of biological evidential materials during the DNA extraction process (Adamowicz *et al*., 2014). This brought the researcher's interest to the application of water-soluble tape as an alternative evidence collection method. It is expected that adhesive on tape can assist efficient collection of trace materials from the deposit surface, and its soluble property would minimize evidence loss on the collection device. Many studies had been using water-soluble tape to collect the bio-evidence such as skin epithelial cells (Li and Harris, 2003; Lempan, 2007), body fluid and stain and fingerprint (Rasmeepaisarn, 2011). From their studies, it was noted that after dissolving the tape, the extraction buffer, became viscous. This made DNA extraction by using silica column-based DNA extraction kit difficult. All of these brought us to the aim of this study to develop an alternative evidence collection method using water-soluble tape-lifts and optimize the protocol to extract DNA from water soluble tape-lifts.

[1] Forensic Science Graduate Program, Faculty of Science, Mahidol University, Ratchathewi, Bangkok 10400
[2] Department of Plant Science, Faculty of Science, Mahidol University, Ratchathewi, Bangkok 10400
[*] Corresponding author: Tel: 0 2201 5232; Fax: 0 2354 7096; E-mail address: nathinee.pan@mahidol.ac.th

2. Methods

2.1 Sample preparation and collection

Reference slides, which cell number was estimated, were prepared from human saliva. Cell number was estimated by cell counting, using haematoxylin and eosin stain, prior to preparation of saliva stain slides. Fresh saliva was diluted with PBS buffer to make slides with 2,000, 1,000, 500, 250, and 100 cells/slide. These were used as control or reference biological samples. Latent fingerprint slides were prepared by pressing 4 fingers against the glass slide for 5 min. These were used to represent biological traces sample.

For collection by tape-lifting method, samples were collected by using a 0.5 x 0.5-cm^2 3M™ Water-Soluble Wave Solder tape 5414 (USA) to repeatingly lift the target sample area for 20 times. Collection by swabbing method was done by wiping the target area for 20 times. These tapes and swabs were put into 1.5 ml Eppendorf tubes for DNA extraction. To compare the extraction methods, samples were collected from the human saliva; including 2,000, 1,000, 500, 250, and 100 cells, and latent fingerprints slides.

2.2 DNA extraction and quantification

DNA samples were extracted by using Chelex method (Walsh *et al*., 1991), modified rapid alkaline extraction method (Watthanapanpituck *et al*., 2014), and DNA IQ extraction kit (Promega, CA, USA) (Promega Corporation, 2013) according to the manufacturer's instructions. DNA samples were quantified by fluorometric assay using Qubit 3.0 fluorometric quantitation kit (Thermo Fisher Scientific Inc., USA) (Thermofisher Scientific, 2014, 2015) according to manufacturer's instruction.

2.3 DNA amplification

PCR was carried out in a total volume of 25 µL, containing 1x PCR buffer, 1.5mM $MgCl_2$, 0.2 mM dNTP, 1unit of Taq DNA polymerase, and 10 pmol of each forward and reverse primer. Thermocycling condition was initial denaturation at 95˚C for 5 min, follow by 30 cycles of denaturation at 60˚C for 30 sec, annealing at 60˚C for 30 sec, extension at 72˚C for 1 min, and final extension at 72˚C for 7 minutes, then hold at 25˚C. Sequences of the 2 primer pairs were as following; mitochondrial DNA human cytB F: [5'-ATC TGA GGA GGC TAC TCA GTA GAC A-3'] and human cytB R: (5'-ATC GGA ATG GGA GGT GAT TCC TAG G-3'); nuclear CSF1PO forward and reverse primers which were designed upstream region of the repeats. Expected PCR product sizes were 160 and 156 bp, respectively (Panvisavas, 2004; Swango *et al*., 2006; Roumruk and Panvisavas, 2014). PCR products were separated and detected in a 3% (w/v) ethidium bromide-stained agarose/Tris-borate-EDTA (TBE) gel.

2.4 PCR inhibitory test

To investigate if there were PCR inhibitory effect of the collection device, water in the amplification reactions were substituted with blank extracts (no DNA extracts) of water-soluble tape and cotton swab. Amplification of the 156-bp CSF1PO marker was carried out as previously described (Roumruk and Panvisavas, 2014), using 60 ng of human gDNA as template.

3. Results and discussion

3.1 Microscopic examination of epithelial cells in saliva and fingerprints

For microscopic examination, epithelial cells in saliva and fingerprint were stained by H&E staining. Cells in saliva were different from fingerprint, both morphology and number. Number of cells in saliva was abundant (ca. 200 cells/µL), and can be diluted to prepare slides with cells between 100 to 2,000 cells per stain. For latent fingerprint, it had 250 cells per print. As showed in Fig. 1, epithelial cells from saliva were intact nucleated cells. In contrast, no nucleated epithelial cells were detected from fingerprint. Results clearly evidenced the lack of nucleated cells in latent fingerprints.

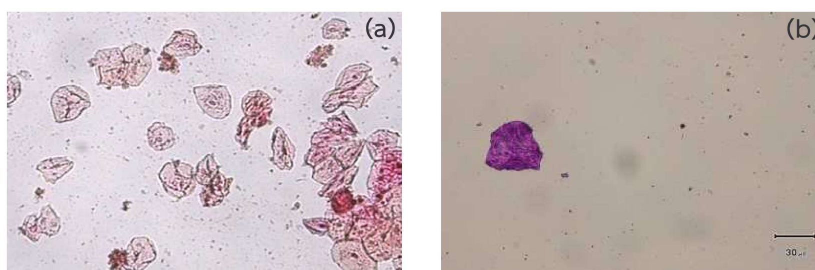

Fig. 1. Comparison of epithelial cells from saliva and latent fingerprint (a, nucleated intact epithelial cells in saliva; b, non-nucleated epithelial cell in fingerprint).

3.2 DNA recovery from saliva and fingerprint using water-soluble tape and swab

Experiments were carried out in triplications. Total amounts of DNA recovered from saliva stain and fingerprint collected by 2 collection methods, i.e., water-soluble tape and swab, which each group were extracted by 3 extraction methods, were estimated. As showed in Fig. 2, total DNA in samples collected by water-soluble tape was higher than swab when extracted by Chelex and rapid alkaline lysis method, but opposite for samples extracted by DNA IQ kit. For DNA recovery from the latent fingerprint, samples were collected from 4 latent fingerprints. Previous experimental results demonstrated that there were ca. 250 cells per 1 print. Therefore ca. 1,000 cells would then be presented in the area of 4 prints. Results showed that total DNA recovered from the fingerprint sample was closed to results of the 1,000 saliva cells/slide. Results of total DNA yield obtain from fingerprint samples could be contributed from human mitochondrial DNA and fragmented nuclear DNA remain. Results showed that Chelex extraction of water-soluble tape lifting method gave the highest amount of total DNA recovered from saliva and fingerprint samples. For the cotton swab collection method, recovery of DNA by Chelex extraction also gave the highest amounts of DNA.

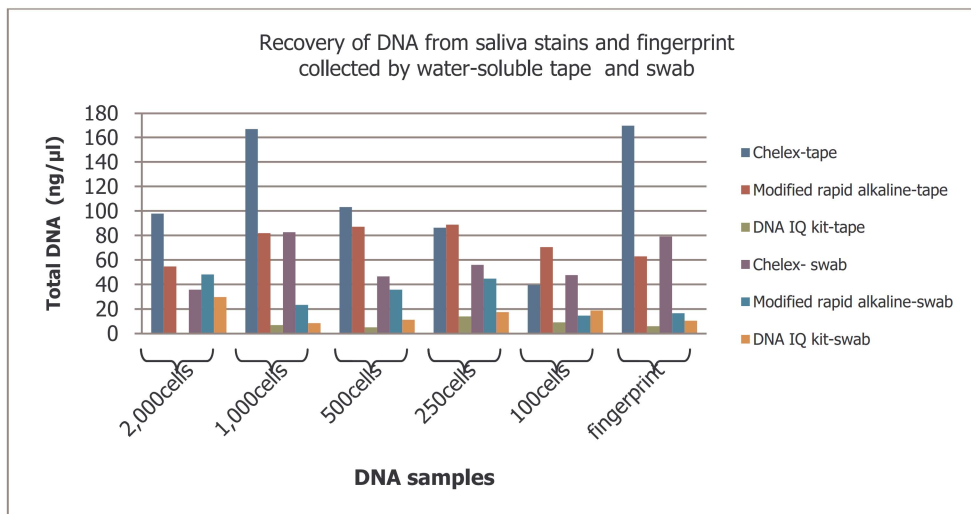

Fig. 2. Total DNA recovered from saliva and fingerprint samples by 2 collection methods, i.e., water-soluble tape-lift and cotton swab, and further extracted by 3 different DNA extraction methods, i.e., Chelex method, modified rapid alkaline lysis method, and DNA IQ extraction kit.

3.3 PCR amplification of the recovered DNA samples

DNA samples including; all dilutions of saliva samples and latent fingerprints were recovered by the 6 combinations of collection and extraction methods were further tested by PCR using both mitochondrial and nuclear DNA markers. As showed in Fig. 3, amplification products of the human cytB and nuclear CSF1PO DNA markers were 160 and 156 bp, respectively.

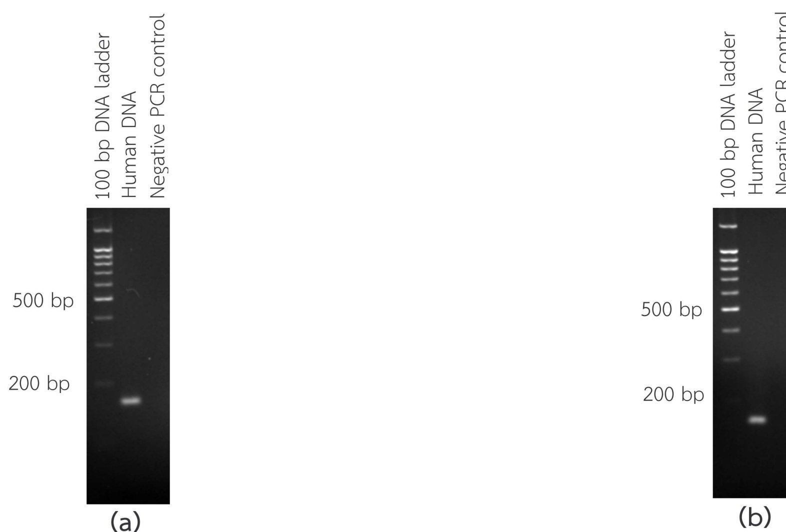

Fig. 3. Photographs of 3.0% (w/v) ethidium bromide stained agarose/TBE gels depicting PCR amplification products of the 160-bp mitochondrial human cytB (a) and the 156-bp nuclear CSF1PO (b) DNA fragments.

PCR analysis results using the 2 DNA markers are summarized in table 1. The 160-bp human cytB mtDNA marker was present in all dilutions of saliva DNA samples recovered by swab method, regardless of the extraction method used. For the water soluble tape collection method, the 160-bp human cytB PCR product was present in all samples except the samples recovered by DNA IQ extraction. PCR analysis of the 156-bp CSF1PO nuclear DNA marker showed that all saliva samples recovered by Chelex and rapid alkaline lysis method gave positive test results, regardless of the collection method. However, for the DNA IQ extraction method, only the saliva sample collected by swab method gave a positive test result. No PCR products of the 156-bp CSF1PO nuclear DNA marker was obtained in fingerprint samples collected by water-soluble tape-lift. This may be due to the absence of nucleated cells in latent fingerprint samples (as described in previous section). The nucleus maybe damaged or lost, and the DNA content could possibly be highly damaged or fragmented. Therefore, no PCR product was obtained when analysed by the CSF1PO primers. The amount of DNA recovered by DNA IQ extraction kit was limited to the surface of the magnetic beads used. In this case, total DNA recovered by this method may be too low for gel-based analysis. In addition, elution volume use in DNA IQ extraction kit maybe further investigated. Using small elution volume will make the DNA extracts become more concentrated and suitable for DNA typing.

Table 1 Summary of PCR amplification results.

Extraction method	Chelex method				Modified rapid alkaline method				DNA IQ extraction kit			
Biological sample	Saliva		Fingerprint		Saliva		Fingerprint		Saliva		Fingerprint	
Collection tool	Swab	Water-soluble tape	Swab	Water-soluble tape	Swab	Water-soluble tape	Swab	Water-soluble tape	Swab	Water-soluble tape	Swab	Water-soluble tape
DNA markers												
1. 160-bp human cytB mtDNA fragment	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	✓	x
2. 156-bp CSF nDNA fragment	✓	✓	x	x	✓	✓	x	x	✓	x	x	x

Furthermore, the PCR inhibitory testing was conducted to demonstrate if any composition used in the DNA recovery method would inhibit PCR amplification by adding blank extracts (no DNA) of water-soluble tape and swab to the PCR instead of water. Amplification results using 60 ng of human gDNA as a template showed that there was no inhibition.

4. Conclusion

It was demonstrated that water-soluble tape can be used as an alternative evidence collection method for collecting biological evidence. There was no inhibitory effect on PCR when using the water-soluble tape as a collection device. The efficiency of this DNA recovery method depends on the nature of cells in sample and DNA extraction method applied. For this study, Chelex method gives the highest DNA yield. This extraction method is simple and inexpensive. Further STR typing experiments on these DNA extracts would help confirm if these methods would be promising for future application in forensic evidence collection.

5. References

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