O-BS004

DNA Recovery from Water-soluble Adhesive Tape

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Abstract

Water-soluble adhesive tape has been generally used, as an alternative of cotton swab, for recovering forensic evidences. This study was aimed to investigate appropriate size of the water-soluble adhesive tape and extraction method suitable for human DNA profile analysis in forensic cases.

Two and a half square centimetres of water-soluble adhesive tape was initially tested for collecting samples from various sources (dried blood, dried saliva, and latent finger prints). Sample DNA were separately extracted by Chelex® 100, QIAamp® DNA Micro kit and QIAamp® DNA Mini kit. No DNA was recovered from QIAamp® DNA Micro kit extractions verified by loci THO1 using agarose gel electrophoresis. In comparison, full 15 STR DNA loci, analyzed by AmpF*l*STR® Identifiler™ and PCR Amplification kit, were obtained from samples of saliva and blood extracted by the QIAamp® DNA Mini kit. However, the latent fingerprint sample produced only partial DNA profile which could be little improved by low copy number (LCN) PCR analysis. Decrease surface area of the tape to 0.04 square centimeter still produced similar results indicating DNA samples in the initial experiments were recovered completely by the tape.

DNA quantification by NanoDrop spectrophotometer was also investigated. There was no correlation between DNA concentration and OD_{260} in the solutions extracted by all the 3 methods suggesting unsuitability of the NanoDrop spectrophotometer in this protocol.

These results provide information for reliable protocol to recover forensic samples by water–soluble adhesive tape for DNA profile analysis. The method is more advantageous than the commonly used cotton swab as DNA extraction from the tape is simple and convenient.

Keywords: Water-Soluble tape, tape-lift, DNA extraction, STR, DNA profile

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Introduction

The major objective of forensic science is to link the evidences at the crime scene to suspects and victims. DNA identification, one of the powerful evidence, has been developed since 1985 by A. Jeffrey, so-called DNA profiling or DNA typing (Jeffreys, Wilson, & Thein, 1985) by amplifying some parts of specific DNA. On the other hand, cotton double swab technique has been commonly used for recovery of forensic sample DNA (Sweet, Lorente, Valenzuela, Lorente, & Alvarez, 1996). However, double swab technique has some disadvantages such as, the cotton have to be cut into small pieces for DNA extraction. Moreover, two swabs, wet and dry swabs are required for optimum DNA recovery (Pang & Cheung, 2007).

In 1994, Chable J et, al. proposed sample collection by a water-soluble adhesive tape which completely soluble in water (Chable, Roux, & Lennard, 1994). The evidence attached on the tape can recuperate by normal water. Tape-lift method is less invasive than cotton swab when collect DNA from donor (Li & Harris, 2003). Adhesive tape lift has been used as an alternative method to recover DNA from blood, tissues, hair and epithelial cells (Zamir, Oz, Wolf, Vinokurov, & Glattstein, 2004 In 2007, Complete DNA profiles collected from biological evidence on clothing have been demonstrated (Lempan, 2007). In general, 10 cm^2 of the tape has been applied for sample collection followed by silica membrane column (QIAamp® DNA Mini Kit) for DNA extraction. Other simpler and less expensive methods, such as Chelex® 100 , QIAamp® DNA Micro Kit , have been claimed not suitable for DNA extraction from the tape interference of the tape components (Lempan, 2007).

Objectives

This study was aimed to examine suitable size of water-soluble adhesive with enough capacity to recovered DNA and complete DNA profile analysis could be obtained and investigated efficiency of common DNA extraction methods for extracted DNA from this tape.

Research Methods

DNA samples

DNA samples were prepared from dry blood, dry saliva and latent fingerprint. Generally, 1 × 2.5 cm^2 of water soluble adhesive tape (Scotch™ 3M, N˚ 5414) were used to recover by tape-lift method. The pressing-lifting cycle was repeated for each sample (up to five times). Then recovered tapes were fold and stored separately in 1.5 ml microcentrifuge tubes at room temperature until DNA extraction.

DNA extraction

1. Chelex® 100

The extraction procedure was performed using Walsh's protocol (Walsh, Metzger, & Higuchi, 1991). One milliliter of sterile water was added into the 1.5 ml micro centrifuge tube containing the adhesive tape vortexed for 10 second followed by incubation at 56˚C for 30 min. The suspension was centrifuged at 13,000 rpm for 2 min. and 900 µl of supernatant was discarded The remaining solution (about 100 µl) was added with 200 µl of 5% Chelex® 100 (BIO-RAD) and 20 µl of Proteinase K (QIAGEN). The solution was mixed by vortex and further incubated at 56˚C for 30 min and 100˚C for 8 min respectively. After centrifugation for 5 min, 250 µl of the supernatant was collected and stored at -20˚C until use

2. QIAamp® DNA Micro Kit

The extraction was followed blood and body fluid spin protocol (QIAGEN, 2007). Two hundred microlitre of sterile water was added into micro centrifuge tube containing the adhesive tape followed by 200 µl of Buffer AL, and 20 µl of Proteinase K (QIAGEN) then mixed by vortex for 10 second and incubated at 56˚C for 10 min. The mixture was added 200 µl of absolute ethanol and vortexed for 10 sec. The lysates were transfer to QIAamp MinElute column and centrifuged at 8,000 rpm for 1

min. Spin columns were changed into a clean 2 ml collection tubes and discarded the tube containing the filtrate. The spin column was added 500 µl of Buffer AW1 then centrifuged at 8,000 rpm for 1 min and placed the spin columns in a new clean 2 ml collection tubes, discarded the tube containing the filtrate. After that 500 µl of Buffer AW2 were added to the spin columns and centrifuged at 13,000 rpm for 3 min followed by discarded the collection tube and replaced with a new 1.5 ml micro centrifuge tube. Finally 100 µl of sterile water were added and incubated at room temperature for 1 min then centrifuged at 8,000 rpm for 1 min. The passed through solution were collected and stored at -20^{o}C.

3. QIAamp® DNA Mini Kit

This protocol was same as QIAamp® DNA Micro Kit method except the spin column was changed from MinElute column to QIAamp Mini spin column.

DNA quantification

DNA concentration was quantified by absorbance measuement using Nanodrop spectrophotometer and NanoDrop 1000 3.7 computer software through Beer's Law. Sterile water was used as blank.

DNA amplification

The existence of DNA after extraction was monitored by presumptive test of the amplified Short Tandem Repeat (STR) loci THO1 . The PCR reaction mixture contained final volume 12.5 µl of 10x PCR buffer (Applied Biosystems) 1.25 µl, 1.5 mM $MgCl_2$ (Applied Biosystems) 1.5 µl, 0.2 mM dNTPs (Applied Biosystems) 0.2 µl, 20 pmole of THO1 primer forward (VIC-GTGATTCCCATTGGCCTGTTC) and reverse (ATTCCTGTGGGCTGAAAAGCTC) for 1 µl per each, 5U AmpliTaq Gold DNA polymerase (Applied Biosystems) 0.2 µl, DNA template 1 µl and sterile water 6.25 µl. The PCR condition was 95ºC 11 min, (95ºC 1 min, 62ºC 1 min, 72ºC 1 min) × 28 cycles, and 60ºC 60 min.

DNA profile analysis was performed using AmpF*l*STR® Identifiler™ PCR Amplification Kit (Applied Biosystems) with final volume 12.5 µl containing 5 µl of PCR reaction mix, 2.5 µl Identifiler primer set, 5U Amplitaq Gold-polymerase (Applied Biosystems), 1 µl of DNA template and sterile water 4 µl. 5 µl without

water was loaded in the sample from latent fingerprint. PCR condition was 95ºC 11 min, (95ºC 1 min, 59ºC 1 min, 72ºC 1 min) for 28 cycles and 60ºC 60 min. LCN analysis, number of cycles were increased to 34 cycles

DNA analysis

The PCRamplified DNA was presumptively detected by agarose gel electrophoresis. The mixtures were contained 10 µl of amplified DNA with 1 µl of 10x loading dye. 10 µl of the mixture were loaded into 2% w/v agarose gel and stained with ethidium bromide. The electrophoretic separation was performed with current 65 V, 500 mA ,60 min. Finally, the gel was visualized under UV lamp and the pictures taken by G:BOX (SYNGENE, UK).

DNA profile analysis was performed using 1 µl of Identifiler's PCR product mixed with 10.7 µl of Hi-Di™ Formamide (Applied Biosystems, UK) and 0.3 µl of GeneScane™ – 500 LIZ® Size Standard (Applied Biosystems). The mixture was separated by capillary electrophoresis through ABI PRISM® 310 Genetic Analyzer with Data Collection Software Version 3.1.0. DNA profiles were analysed by GeneMapper™ *ID* Software Version 3.2 on ABI PRISM® 310 Genetic Analyzer (Applied Biosystems).

Results

DNA recovery

Water-soluble adhesive tape size 1 × 2.5 cm^2 was able covered whole 20 µl of dried blood and saliva and able to recovered most of dried blood but dried saliva was still stained on surface.

DNA quantification

OD_{260} from samples which extracted by Chelex® 100 method were 0.703 - 1.497 while, OD_{260}/OD_{280} were 0.888-0.896 however, negative control was also absorbed light too. And calculated DNA concentration by Beer's Law were 23.7 – 53.6 ng/µl. On the other hand, OD from Mini kit extraction was lower than Chelex® 100, 1.00 – 1.54 for OD_{260} and 1.824 – 2.367 for OD_{260}/OD_{280}. Calculated DNA concentration were 4.98 – 7.68 ng/µl.

DNA extraction

The solution were blocked inside column when used 1 × 2.5 cm^2 of tape and extracted by Micro kit column and there were yellow gel membrane above silica membrane. However, the tape size 1 × 2.5 cm^2 was not blocked when extracted by Mini kit method (Figure 1).

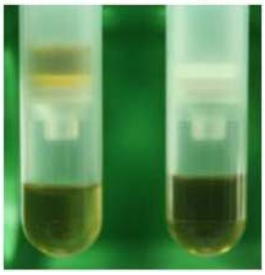

Figure 1. Comparison of spin column's blocking. Left is Micro column. Right is Mini kit.

DNA analysis

Presumptive amplification DNA were detected by Agarose gel electrophoresis. Only dried blood were detected when extracted by Chelex® 100 method (Data not shown) while all samples from dried and dried saliva which extracted by QIAamp DNA Mini kit were detected DNA loci THO1 but DNA from latent fingerprints were not detected (figure 2).

Complete STR DNA profiles were obtained from 1 × 2.5 cm^2 water-soluble adhesive tape which recovered dried blood and dried saliva and extracted by QIAamp® DNA Mini Kit, 16 from 16 STR loci (Figure 2). DNA profiles from latent fingerprint were partials with 4 loci matched from 16 loci (data not shown). Therefore LCN was performed for DNA from latent fingerprint and number of DNA loci were increase to 8 (Figure 3). Average peak height from dried blood samples were higher than dried saliva and latent fingerprint respectively (table 1).

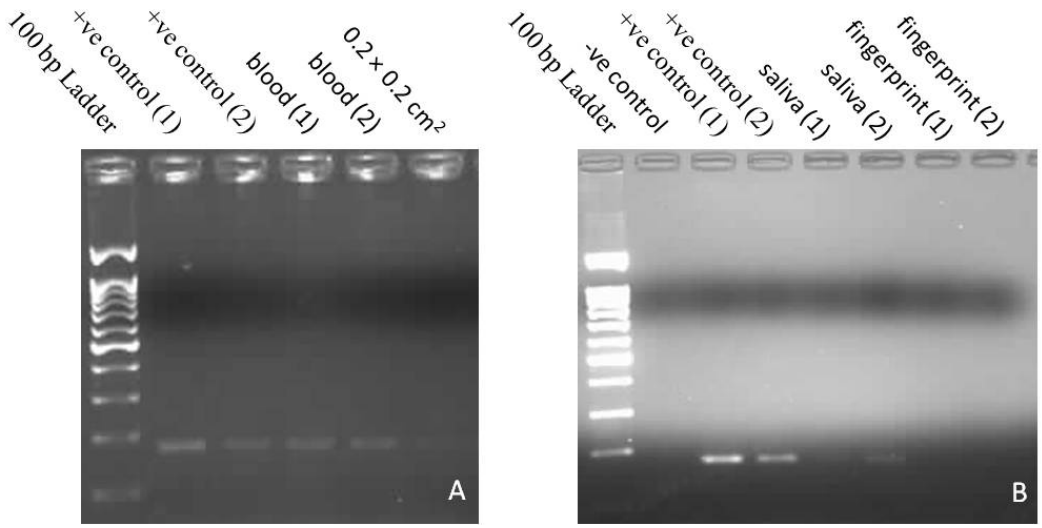

Figure 2. Amplification product of presumptive test at loci THO1 which extract by QIAamp® DNA Mini Kit on 2% w/v agarose gel. Positive control (1) is fresh blood and (2) is buccal swab. A) 20 µl of dried blood recover by 1 × 2.5 cm^2 and 0.2 × 0.2 cm^2 of water-soluble adhesive tape. B) 20 µl of dried saliva and latent fingerprint recover by 1 × 2.5 cm^2 of tape.

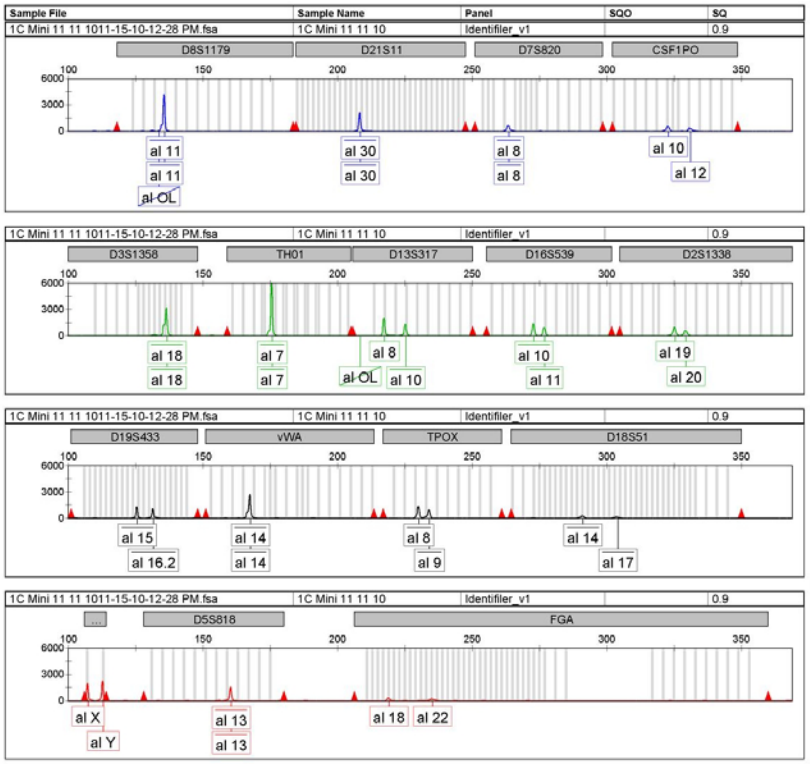

Figure 2. Complete STR DNA loci from 20 µl of dried blood which recover by 1 × 2.5 cm^2 and extract by extract by QIAamp® DNA Mini Kit.

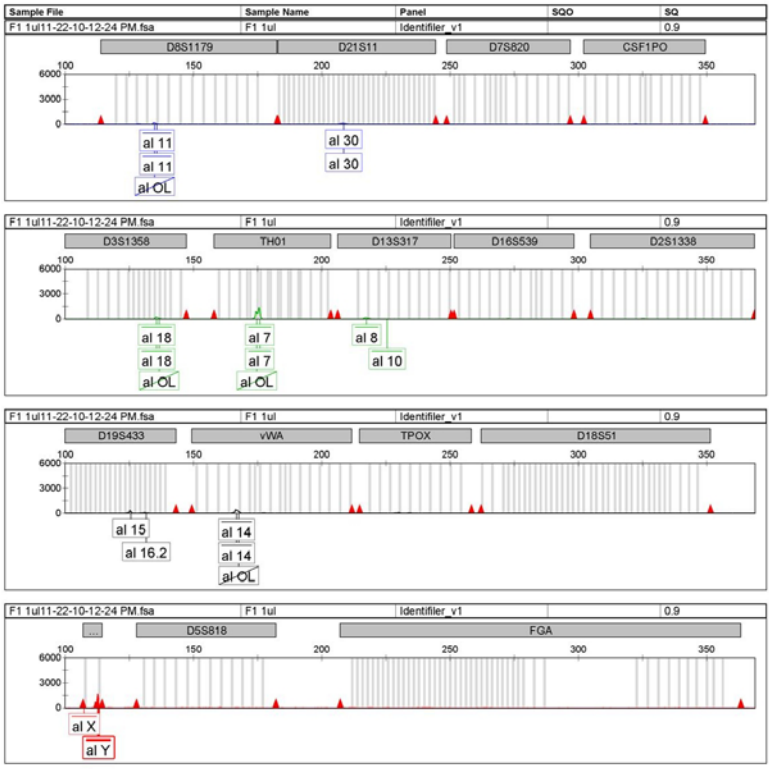

Figure 3. Partial STR DNA loci from 20 µl of latent fingerprint which recover by 1 × 2.5 cm^2 and extract by extract by QIAamp® DNA Mini Kit.

Sample	No. of allelic peaks (complete = 32)	Mean peak height (rfu)
Blood (1)	32	1268.32
Blood (2)	32	1313.88
Saliva (1)	32	363.28
Saliva (2)	32	677.37
Fingerprint (1)	16	372.81
Fingerprint (2)	16	439.50
Blood 0.2×0.2 cm^2	32	1229.25
+ve control (1)	-	-
+ve control (2)	32	1228.16
-ve control	0	0

Table 1. Summary DNA profiles from QIAamp® DNA Mini Kit method. All samples are recover by 1×2.5 cm^2 of tape except sample blood 0.2×0.2 cm^2, and control.

Discussion and Conclusion

Water-soluble adhesive tape with tape-lift method was easy and effective to recovered DNA on smooth surface. The tape size 1×2.5 cm^2 had enough capacity to recovered DNA and obtained complete STR DNA profile. The components from this size of tape was not interfered QIAamp® DNA Mini Kit due to tape solution was not blocked inside spin column. However, the solution was still blocked inside Micro kit's column. Reducing size of tape was reduced components from tape itself which interfered DNA extraction. For dried blood, the tape could reduced to 0.04 cm^2 and still obtained complete profile

This study could not specify quantity of recovered DNA because spectrophotometer was not suitable for measured DNA extracted from water-soluble adhesive tape because there was no correlation between DNA concentration and OD_{260}. The tape's components were able to interfered light absorbance and leaded to

error for measured DNA. Because of Chelex® 100 procedure was not include a purification step (Clark). However, QIAamp® DNA Mini Kit were also approached the same problem due to similar of light absorbance but different of peak height. This might explained by QIAamp® DNA Mini Kit the lysate buffering conditions are adjusted to allow optimal binding of the DNA to the QIAamp silica-membrane (QIAGEN, 2007) but there was contaminant from tape which able to avoid washing and eluted from membrane by water and contaminated DNA samples.

Complete of DNA profile from dried blood, dried saliva but amount of DNA were different. This was because DNA in saliva was lower than blood, concentration of the DNA extract from 50 µl of blood was 25 ng/µl while 50 µl saliva was 2 ng/µl (Castella, Dimo-Simonin, Brandt-Casadevall, & Mangin, 2006). Including nature of blood when dried was easier to release from surface than dried saliva, therefore, the tape was able to recovered more DNA from dried than dried saliva. The amount of DNA from latent fingerprint was too low to amplified for complete STR DNA profile. Low copy number (LCN) analysis was able to improve number of STR loci detected. The STR loci detected were increased from 4 from 16 STR loci from normal PCR (data not shown) to 8 from 16 STR loci. However, stutter, shoulder peak and allele drop in-out were occurred for LCN analysis, thus, the analysis of LCN profile was recommended to compared with reference DNA profile. Nevertheless, DNA on latent fingerprint was concerned many factors such as 1) good or bad shedders, 2) hand washing, 3) secondary transfer (Phipps & Petricevic, 2007).

This study shows that 1 × 2.5 cm^2 water-soluble adhesive tape with tape-lift method and extract DNA by QIAamp® DNA Mini Kit is a efficiency DNA recovery method for blood. Complete DNA profile can be obtained from tape without PCR inhibiter. This recovery method is able to reduce the risks of DNA degradation due to bacteria action, moisture and air, which are encountered using conventional collection methods and stable for one month after collection (Li & Harris, 2003). NanoDrop spectrophotometer is not suitable for quantify DNA from water-soluble adhesive tape because contamination from tape's component.

Suggestions

Exact quantity of DNA recover by water-soluble adhesive tape, long-term storage, and comparation efficiency of DNA recovery to traditional collection method should be studied in the future experiments.

References

Castella, V., Dimo-Simonin, N., Brandt-Casadevall, C., & Mangin, P. (2006). Forensic evaluation of the QIAshredder/QIAamp DNA extraction procedure. *Forensic science international, 156*, 70-73.

Chable, J., Roux, C., & Lennard, C. (1994). Collection of fiber evidence using water-soluble cellophane tape. *Journal of forensic sciences, 39*, 1520-1520.

Clark, L. Chelex® 100 Extraction. *Advantages and Disadvantages*, http://www.nfstc.org/pdi/Subject03/pdi_s03_m03_01_a.htm (Accessed November 14, 2010)

Jeffreys, A., Wilson, V., & Thein, S. (1985). Hypervariable'minisatellite' regions in human DNA. *Nature, 314*(6006), 67-73.

LEMPAN, A. (2007). *DNA RECOVERY FROM FORENSIC CLOTHING SAMPLES BY TAPE-LIFT*. MAHIDOL UNIVERSITY.

Li, R. C., & Harris, H. A. (2003). Using hydrophilic adhesive tape for collection of evidence for forensic DNA analysis. *J Forensic Sci, 48*(6).

Pang, B., & Cheung, B. (2007). Double swab technique for collecting touched evidence. *Legal Medicine, 9*(4), 181-184.

Phipps, M., & Petricevic, S. (2007). The tendency of individuals to transfer DNA to handled items. *Forensic science international, 168*(2-3), 162-168.

QIAGEN. (2007). *QIAamp® DNA Mini and Blood Mini Handbook 2nd edition*. CA, USA.

Sci, J. (2003). Using hydrophilic adhesive tape for collection of evidence for forensic DNA analysis. *J Forensic Sci, 48*(6).

Sweet, D., Lorente, M., Valenzuela, A., Lorente, J., & Alvarez, J. (1996). Increasing DNA extraction yield from saliva stains with a modified Chelex method. *Forensic science international, 83*(3), 167-177.

Walsh, P., Metzger, D., & Higuchi, R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, *10*(4), 506.

Wickenheiser, R. (1992). Fibre Concentration by Membrane Vacuum Filtration in Preparation for Rapid Microscopic Comparison. *The Canadian Society of Forensic Science Journal*, *25*(3), 177-l.

Zamir, A., Oz, C., Wolf, E., Vinokurov, A., & Glattstein, B. (20004). A Possible Source of Reference DNA from Archived Treated Adhesive Lifters. *J Forensic Sci*, *49*(1).