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TECHNICAL NOTE

Richard C. Li,[1] *Ph.D. and Howard A. Harris,*[2] *Ph.D., J.D.*

Using Hydrophilic Adhesive Tape for Collection of Evidence for Forensic DNA Analysis*

ABSTRACT: Known exemplar samples of human DNA have traditionally been body fluids, such as blood, saliva, and semen. In each case, the presence of water is a risk for the bacterial growth, which may degrade the DNA evidence. In this study, the authors have developed a method that employed a hydrophilic adhesive tape (HAT) for collecting DNA evidence. The HAT method was used to remove surface cells from relatively hairless areas on the body. The area examined were ankle, arm, behind the ear, between fingers and back of the neck. The HAT was then dissolved in the extraction buffer. DNA typing was performed at vWA, TH01, F13A1, and FES loci using the short tandem repeat (STR) analysis. Our results show that the samples collected from ear give the best results with a success rate of 100%. All subjects tested by this method had known STR genotypes established from buccal swabs. The authors' results suggest that the HAT method can be used as a less invasive method for collecting biological evidence for forensic DNA analysis. In addition, this collection method should reduce the risk of DNA degradation due to the moisture, which is encountered using conventional collecting methods.

KEYWORDS: forensic science, STR, DNA evidence collection, tape lifting

Traditionally, body fluid samples have been used as DNA control samples. In the early days of restriction fragment length polymorphism (RFLP) analysis, blood was the material of choice (1,2). As the technology has evolved, buccal swabs have become an attractive alternative (3,4). Both blood and the saliva associated with buccal samples have the disadvantage of being fluid based and therefore susceptible to degradation from bacterial contamination (1). A relatively new method for collection is the use of FTA paper (5). FTA paper is an absorbent cellulose-based paper that contains chemicals to protect DNA molecules from nuclease degradation and preserve the paper from bacterial growth (6). However, the collection of body fluids is somewhat invasive of the donor's person and under most circumstances a court order is a constitutionally mandated requirement. Although the buccal swab is less invasive, it still requires the cooperation of the individual being sampled and is much more likely to be subjected to external contamination. In addition, the above mentioned techniques provide samples that must either be processed or dried before they can be safely stored for a significant period.

In this study, the use of hydrophilic adhesive tape (HAT) allows significantly less invasive sampling than even a buccal swab because it requires only pressing the sticky tape against the skin in a hairless location. By placing the two sticky sides together the sample is protected from contamination, moisture, and most other possible environmental insults. A portion of this tape can be cutoff and dissolved directly in extraction buffer. The resultant solution can be sampled for quantitative analysis of the nuclear DNA present and then an appropriate aliquot taken for amplification and subsequent DNA analysis (see below, sampling procedure).

Recent studies reported successful DNA typing from material adhered to regular adhesive tapes associated with the collection of gun shot residue and fingerprints (7,8). In our study, we employ a HAT method for efficient collection of DNA control samples. The HAT strip could be completely dissolved during DNA extraction process, thus we speculate that the yield of DNA using HAT method should be higher than that of regular adhesive tape method. Our preliminary work was reported previously (9).

Methods

Sampling Procedure

Surface cells from the body area to be sampled were collected with a piece of the HAT (Scotch™, Wave Solder Tape 5414; approximately 3 × 6 cm). The sampling procedure was performed by firmly pressing the tape to the skin followed by lifting it free of the skin. The pressing-lifting cycle was repeated for each sample (up to eight times) until the tape seemed to no longer adhere to the skin surface when pressed against it. The translucent adhesive tape clearly showed the area of the tape that had contacted the skin. The tape was then folded over onto itself and stored in a sealed plastic bag at room temperature until needed for DNA extraction. Gloves were worn to eliminate any contamination from the individual doing the sampling. All items (except gloves) used

[1] Forensic Science Program, College of Criminal Justice, Sam Houston State University, Huntsville, TX.
[2] Forensic Science Program, School of Public Safety, University of New Haven, West Haven, CT.

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for sampling were cleaned with 70% ethanol solution prior to the sampling.

DNA Extraction

A strip of HAT (between 1 × 1 and 1 × 2 cm) was cut for DNA extraction. This small tape portion was placed in a microcentrifuge tube (1.5 mL). DNA was extracted by using Chelex® extraction method (10). Two hundred μL of Chelex® 100 (Bio-Rad Laboratories) solution (Tris, pH 8.0, 1 mM; EDTA, 0.01 mM; Chelex® 100, 5%) was used and the tape-buffer solution was heated at 56°C for 20 min and then at 100°C for 8 min followed by vortexing. The DNA extracts were then stored at −20°C. The DNA yield extracted from the ear area was estimated at approximately 10 ng/200 μL range.

Short Tandem Repeat (STR) Analysis

Four loci multiplex analysis—Up to 27 μL (approximately 14%) of the total extract (200 μL) was used for DNA amplification. The fragments at loci vWA (11), TH01 (12), F13A1 (13), and FES (13) were amplified. Polymerase Chain Reaction was performed according to the procedures of Kimpton et al. (14) and Applied Biosystems (15).

The COfiler™ and Profiler Plus™ systems (Applied Biosystems)—Polymerase Chain Reaction was performed according to the procedure of Applied Biosystems (15).

The amplified product was analyzed using an ABI PRISM® 377 Genetic Analyzer (4% denatured polyacrylamide gels) and an ABI PRISM® 310 Genetic Analyzer respectively.

Results and Discussion

The Effect of Dissolved Tape Solution on PCR Reaction

An experiment was designed to determine the amount of tape which could be employed for collection of DNA and which would not inhibit PCR amplification. A square 1 × 1 cm of the tape was cut and dissolved in 200 μL TE buffer (Tris, pH 8.0, 1 mM; EDTA, 0.01 mM). Amplification was carried out with an increasing volume of the tape solution (0, 10%, 20%, 30%, 40% and 50%, respectively) added to the reaction mix (total 25 μL). The peak height of TPOX locus was analyzed. The results demonstrated that no reduction of peak height was observed at TPOX locus when adding the dissolved HAT solution in proportions of 10–50% of the final volume of PCR reaction mix (see Fig. 1). This result indicates that no inhibition of DNA amplification was observed from adding the dissolved tape solution in proportions of 10–50% of the final volume. In fact, there appears to be a small increase in yield after amplification in the samples that had the tape solution added. Since the main concern was the possibility of inhibition by the tape, this small effect was not further explored at this time.

The Effect of Area of the Collection on DNA Typing

Surface cells from several different, relatively hairless, areas of the body were collected using the HAT method. The areas examined were: 1) ankle, 2) arm inside elbow (crook of arm), 3) behind the ear, 4) between fingers, and 5) back of the neck. Our results show that the genotype profile analysis could be performed from the samples collected from the ear, finger, neck and arm. In contrast, no alleles were detected from the samples collected from the ankle area. The overall success rate for STR analysis was de-

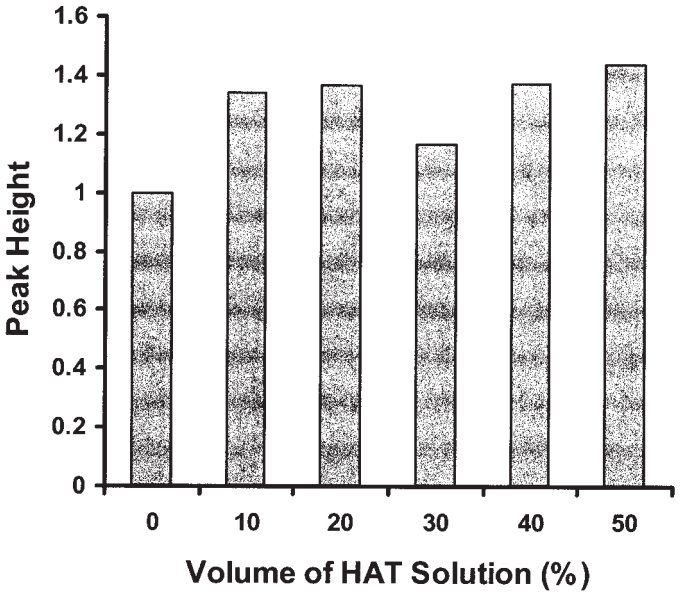

FIG. 1—*The effect of dissolved HAT solution on PCR reaction. Amplification was carried out with an increasing volume of the HAT solution 0, 10%, 20%, 30%, 40% and 50% respectively (25 μL final reaction mix). A 0.5 ng sample of human genomic DNA was used for template DNA. The peak height of TPOX locus was measured and was normalized for loading by measuring the peak height of ROX standard. Y-axis, the peak heights are expressed relative to those of the corresponding PCR reaction without HAT addition. The results shown are the averages of three experiments. The peak height of TPOX locus were observed between 1446 to 3600 RFU (relative fluorescent unit).*

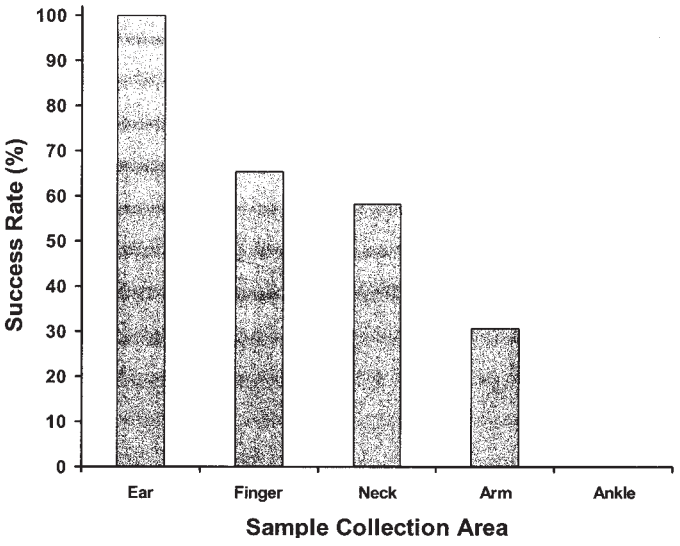

FIG. 2—*The effect of area of the collection on STR analysis and comparison of success rate of STR analysis on four genetic loci. The success rate is presented as the percentage of the number of alleles detected over the number of alleles tested. The success rates for STR analysis at four loci (vWA, TH01, F13A1, and FES) were compared among five body areas (ear, fingers, neck, arm, and ankle). The success rate for STR analysis is presented as the percentage of the number of alleles detected over the number of alleles tested. Heterozygote was treated as two entries and homozygote was treated as a single entry. The success rates are: ear, 100% (13 subjects); fingers, 65.5% (13 subjects); neck, 58.3% (5 subjects); arm, 30.6% (5 subjects); ankle, 0 (5 subjects).*

termined in terms of the percentage of alleles that were successfully detected. The success rate of four-loci multiplex system (vWA, TH01, F13A1, and FES) is: ear, 100% (13 subjects); finger, 65.5% (13 subjects); neck, 58.3% (5 subjects); and arm, 30.6% (5 subjects) (Fig. 2). Among the subjects tested for ear and finger, we included Caucasian (54%), Negroid (23%), Asian (23%), male (77%), and female (23%). The lower success rate observed in finger, neck, arm, and ankle samples is due to the detecting of partial profiles or failure to detect a profile in some of the loci. This is mainly due to the occurrence of a low yield of DNA template and stochastic effect. We think that the amount of cells tape-lifted from these skin areas is lower than that of the ear area thus leads to a low yield of DNA template. A small trial (5 subjects) of ear sample was analyzed by using Profiler Plus™ system. The genotype profiles were successfully identified (Li & Harris, unpublished).

All subjects tested by tape-lift method had known STR allele patterns established from buccal swabs. In all subjects, the STR allele patterns obtained from the ear samples (HAT method) corresponded to the known patterns generated from the buccal swab. One representative STR profile is shown in Fig. 3 in which four loci (vWA, TH01, F13A1, and FES) were tested.

This study shows that the use of HAT provides a less invasive method for collecting DNA control samples and potentially evidence samples for STR analysis. The data demonstrate that DNA profiles can be obtained using HAT without an inhibitory effect on DNA amplification. STR analysis was successfully performed on the surface cells collected from several areas of the body using this HAT method. In addition, the DNA genotype profiles obtained were consistent with those from other DNA sampling techniques such as buccal swab or blood collection. As indicated above, this collection method should reduce the risks of DNA degradation due to bacteria action, moisture and air, which are encountered using conventional collection methods. In addition, our results have demonstrated that using the HAT method, the DNA samples are stable for one month after collection (Li & Harris, unpublished). The stability of the DNA samples under long-term storage should be studied in the future experiments.

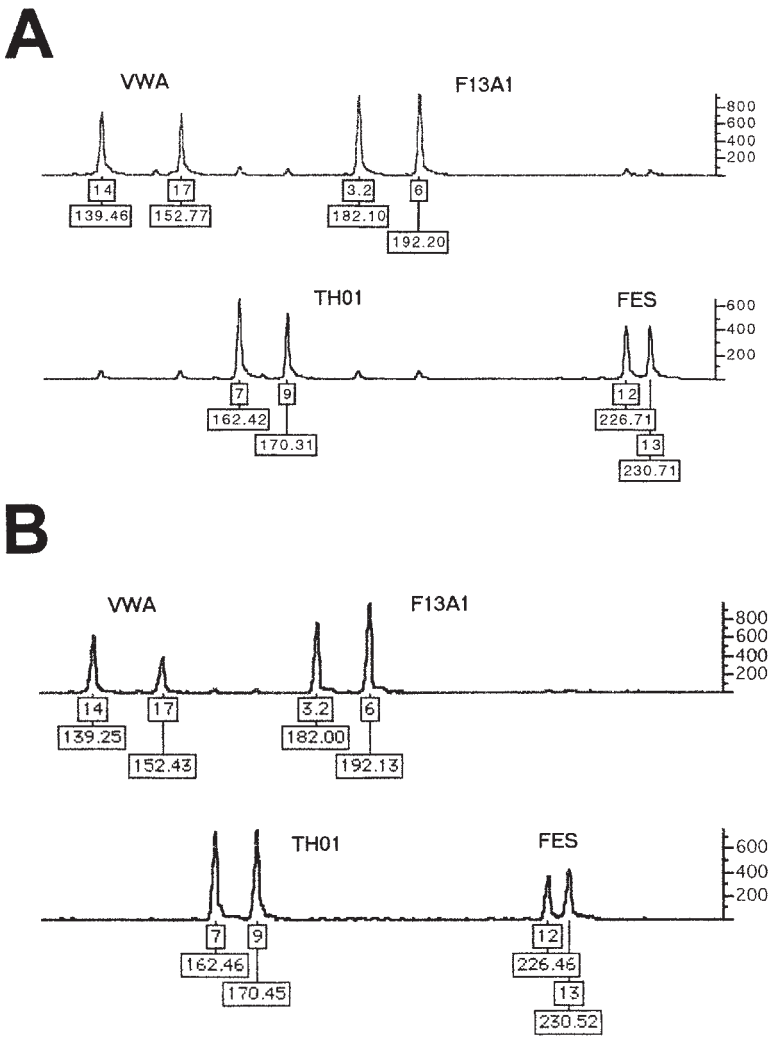

FIG. 3—*Comparison of DNA profiles of buccal swab samples and ear samples collected by HAT method. All subjects tested by HAT method had known STR allele patterns established from buccal swabs. Four loci (vWA, TH01, F13A1, and FES) were analyzed and compared. In all subjects, the STR allele patterns obtained from the ear samples (HAT method) corresponded to the known patterns generated from the buccal swab. One representative STR profile is shown. The Y-axis is expressed as RFU (relative fluorescent unit). A: buccal swab sample; B: ear sample collected by HAT method.*

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Additional information and reprint requests:
Richard C. Li, Ph.D.
College of Criminal Justice
Sam Houston State University
Box 2296
Huntsville, TX 77341-2296
Tel: 936-294-3082
E-mail: cjc_rcl@shsu.edu