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TECHNICAL NOTE

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A Possible Source of Reference DNA from Archived Treated Adhesive Lifters

ABSTRACT: During the course of a double murder trial, it became apparent that the two adhesive lifters from the two cadavers had been mislabeled before being presented in court. The question was raised whether DNA testing from the biological material remaining attached to the lifters could resolve this mix-up.

In fatal shooting cases where a bullet has been fired through a body surface, an adhesive lifter is applied directly to the entrance wound. The total nitrite residues, as well as biological material surrounding the wound (blood, hair, tissue) are transferred to the adhesive lifter. The nitrite residues are used for estimating firing distance. In a worst-case scenario, the biological material on the lifter may be the only remaining reference material from a victim. In this paper, we examined whether the biological material retrieved from adhesive lifters could be used for DNA typing after the lifters had been treated for GSR pattern. In as much as the biological material found on the lifters can be typed and profiled following physical and chemical treatment, we submit that archived adhesive lifters can be used as a future source of reference DNA from cadavers where no other sample is available.

KEYWORDS: forensic science, adhesive lifter, STR typing, silver staining, SGM Plus

The estimation of firing distance is based on the examination of a bullet entrance hole and the gunshot residue (GSR) pattern found around the wound. Gunshot residues are made up of gunpowder and primer residues as well as metal particles from the bullet and cartridge case (1). In most cases there is also a need for a color chemical test to assess the GSR patterns around the entrance bullet hole.

In shooting cases where a bullet is fired directly through a body surface and does not pass through any intermediate medium, as in a fatal head wound, an adhesive lifter is applied directly to the entrance wound (2). The total nitrite residues (nitrite ions, unburned and partially burned smokeless powder residues) are transferred from the target directly to the adhesive lifter. When the adhesive lifter is applied to the wound, biological material in the form of blood, tissue, hairs, or epithelial cells from the wound area is also transferred to the lifter. The gunshot residue pattern of the smokeless powder residues on the adhesive lifter is then visualized by alkaline hydrolysis conducted at a high temperature (100°C), and then the Modified Griess Test (MGT) as a color test for nitrites is carried out (1,3).

In Israel this procedure for shooting distance estimation has routinely been employed on all fatal shooting victims preceding autopsy since 1997, and these adhesive lifters are then archived indefinitely within the casework files. On the other hand, biological materials sampled during autopsy from cadavers are kept for only a period of one year in the National Center of Forensic Medicine unless there is a specific request by the police to keep them longer.

If DNA typing is possible from archived treated adhesive lifters, this may provide a future usable source of reference DNA from subjects where no other sample is available.

In order to address this possibility, we set up a two-stage experimental system to check the feasibility of recovering DNA profiles from previously physically and chemically treated adhesive lifters.

Experimental

Total Nitrite Pattern Visualization from Body Surfaces

Materials—Transparent adhesive lifters ("JAC Vinyl," 25 cm by 25 cm by 80 μm) with a protective cover (supplied by ISA Ltd., Greasley Street, Bulwell, Nottingham, England), 2% KOH in ethanol, Modified Griess Test (MGT) reagent made of 3% Sulfanilamide and 0.3% *N*-(1-naphthyl) ethylenediamine dihydrochloride dissolved in 5% phosphoric acid (AR) and fixed photographic paper.

Procedure—The adhesive lifter is manually applied firmly to the area of the gunshot wound. At this stage, biological material from around the wound is transferred to the adhesive lifter. The location of the entrance hole or the hole shape is marked on the back of the transparent adhesive lifter. The adhesive lifter is then removed from the wound and attached to a silicone cover. In the laboratory the adhesive lifter is removed from the silicone cover, attached to cardboard, and lightly sprayed with the KOH solution. The lifter is then placed in a 100°C oven for 1 h. A desensitized piece of photographic paper is dipped into a MGT reagent solution for a few

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seconds. All excess solution on the photographic paper is removed by wiping with filter paper. This photographic paper is then placed on the adhesive lifter and subjected to a pressure of 1.3 atm in a press at 70°C for approximately 1 min.

DNA Profiling

It has previously been reported that STR profiles can be obtained from samples exposed to extremely high temperatures (4,5). The treatment employed on adhesive lifters for gunshot residue is a combination of a strong base and high temperatures. We were not aware what affect this treatment would have on STR typing.

The first phase of the study was to determine if DNA profiles could be generated from biological material deposited on untreated and treated adhesive lifters. To accomplish this, blood from four laboratory workers was dripped on adhesive lifters and left untreated. This procedure was repeated on another set of adhesive lifters, and these lifters were then submitted to alkaline hydrolysis treatment and the Modified Griess Test (MGT), the method employed for estimation of firing distance from body surfaces, as described above. A small piece (approximately 1 by 1 cm) from each lifter was cut and placed in microcentrifuge tubes. DNA was extracted using a phenol/chloroform extraction method (6). The extracted DNA was then amplified and analyzed using the PCR method for the following short tandem repeat (STR) loci: CSF1PO, TPOX, THO1, F13A, FESFPS, VWA, D7S820, D13S317, and D16S539 using CTT triplex, FFV triplex, and Silver STR III triplex kits (Promega, Madison WI) (7). The amplified products were separated on 4% polyacrylamide gels and visualized by silver staining (8).

The second phase of the experiment consisted of attempting to profile DNA from biological material recovered from archived and treated adhesive lifters that came from actual former casework. Only twelve adhesive lifters, former items of evidence, spanning the years 1997–2000 were available for this purpose. Dried blood was visible on all the lifters in varying amounts from small specks to gross flakes (Table 1). Areas surrounding the area marked as the entrance wounds on the adhesive lifters were cut and placed in microcentrifuge tubes. DNA was extracted as described above, and yields were estimated by visualization on ethidium bromide stained agarose gels.

Although exact quantitation methods of extracted DNA is recommended for accurate determination of template DNA for multi-locus PCR reactions (9,10), such a method was not available in our lab at the time these experimental procedures were carried out. The estimated amounts of extracted DNA were successfully amplified and then analyzed using either the method described above (seven of the twelve samples) or (in five of the twelve samples) by employing the multi-locus amplification AmpF*l*STR SGM Plus kit (Perkin-Elmer Applied BioSystem, Foster City, CA) containing eleven loci (ten STR loci and Amelogenin) (11). Approximately 2 ng of template DNA was amplified with the SGM Plus kit using the GeneAmp PCR Stystem 9700 (Perkin Elmer Applied BioSystems, Foster City, CA). Amplification reactions were carried out according to the manufacturer's instruction. Capillary electrophoresis was performed using the Perkin Elmer Applied BioSystems ABI CE 310 systems, and alleles were defined using the GeneScan and Genotyper 2.5 software (Applied BioSystems, Foster City, CA).

TABLE 1—*DNA profiling from treated adhesive lifters from actual casework spanning years 1997–2000.*

Sample No. and Year	Biological Material Seen on Lifter	PCR Method	Number of Loci Profiled
1886/1997	+++	CTT,DDD,FFV	9/9
7913/1997	+++	CTT,DDD,FFV	9/9
11819/1997	++	CTT,DDD,FFV	4/9
18404/1997	+++	CTT,DDD,FFV	9/9
19881/1997	++	CTT,DDD,FFV	7/9
4352/1998	+	CTT,DDD,FFV	1/9
11156/1998	++	SGM Plus	11/11
14234/1998	+	SGM Plus	10/11
19381/1998	++	SGM Plus	11/11
9296/1999	+++	SGM Plus	5/11
22380/1999	+	SGM Plus	11/11
22841/2000	+	CTT,DDD,FFV	0/9

[+] Indicates that very little biological material/dried blood seen on lifter (seen as specks).
[++] Indicates a moderate amount of biological material/dried blood.
[+++] Indicates greater amounts of biological material/dried blood that were viewed as dried blood flakes, etc.

Results and Discussion

In the first stage of the experiment, DNA profiles matching those of the known donors were obtained from both the treated and untreated adhesive lifters (data not shown). This indicates that the treatment employed for shooting distance estimation, such as strong bases, excessive heating, and employment of acids and other organic materials, is not detrimental to future DNA typing. The second phase of the experiment employed adhesive lifters from actual casework sampled from cadavers following physical and chemical treatment that spanned over a five-year period. Table 1 summarizes the results from the second stage of the experiment. We were able to conclude from our experiment that the amount of time that has passed between sampling/treatment of the adhesive lifters and DNA testing (silver-stain or fluorescent visualization) does not inhibit obtaining a viable DNA profile.

This experiment was originally the result of a question posed from an actual case study. A double murder was carried out by shotgun. Shooting distance estimation was done as described above by the application of adhesive lifters to the site of the gunshot wounds preceding the autopsies of the two victims. During the course of the murder trial it became evident that the adhesive lifters relating to the two cadavers had been switched and erroneously labeled. In this case the mistake was rectified by the physical comparison of the two lifters to the corresponding bullet wounds of the two victims. One victim had one entrance wound, while the second victim suffered two bullet wounds. Here there was no doubt as to which adhesive lifter related to which cadaver, but the question was raised whether confirmation of identity could have been carried out from DNA profiling of the biological material remaining on the adhesive lifters after their physical and chemical treatment employed for shooting distance estimation.

There may arise circumstances where original DNA samples have become exhausted for further forensic analysis. Although the actual purpose of adhesive lifters is not as a bank for archived DNA samples, we saw in filed adhesive lifters a possible source for reference DNA where no other samples are available and may prove priceless in such cases. Identifications in mass disasters, paternity testing, or genetic analysis are just a number of such examples.

In conclusion, archived previously treated adhesive lifters may be used as possible reference samples of DNA in cases where DNA is no longer available from any alternative source.

References

1. Glattstein B, Vinokurov A, Levin N, Zeichner A. Improved method for shooting distance estimation. Part I. Bullet holes in clothing items. J Forensic Sci 2000;45(4):801–6. [PubMed]

2. Glattstein B, Zeichner A, Vinojurov A, Nadav L, Jugel C, Hiss J. Improved method for shooting distance estimation. Part III. Bullet holes in cadavers. J Forensic Sci 2000;45(6):1243–9. [PubMed]
3. Glattstein B, Zeichner A, Vinokurov A, Shoshani E. Improved method for shooting distance estimation. Part II. Bullet holes in objects that cannot be processed in the laboratory. J Forensic Sci 2000;45(5):1000–8. [PubMed]
4. Clayton TM, Whitaker JP, Maguire CN. Identification of bodies from the scene of a mass disaster using DNA amplification of short tandem repeat (STR) loci. Forensic Sci Int 1995;76:7–15. [PubMed]
5. Clayton TM, Whitaker JP, Fisher DL, Lee DA, Holland MM, et al. Further validation of a quadruplex STR DNA typing system: a collaborative effort to identify victims of a mass disaster. Forensic Sci Int 1995;76: 17–25. [PubMed]
6. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Harbor, 1989.
7. Promega Corporation. Gene Print™STR system [technical manual]. Madison, WI: Revised 4/01.
8. Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC. Analysis of the VNTR locus D1S80 by PCR followed by high-resolution PAGE. Am J Hum Genet 1991;48:137–44. [PubMed]
9. Walsh PS, Varlaro J, Reynolds R. A rapid chemiluninescent method for quantitation of human DNA. Nucleic Acids Res 1992;20:5061–5. [PubMed]
10. Nicklas JA, Buel E. Development of an *Alu*-based, QSY 7-labeled primer PCR method for quantitation of human DNA in forensic samples. J Forensic Sci 2003;48:282–91. [PubMed]
11. The Perkin-Elmer Corporation. AmpFlSTR SGM Plus™ [user's manual]. Foster City (CA): Applied Biosystems, 1999.

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