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TECHNICAL NOTE

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Successful Extraction of Human Genomic DNA from Serum and Its Application to Forensic Identification

ABSTRACT: We report here on the successful extraction of human genomic DNA from a serum sample in a forensic case. The extracted DNA was successfully used for the identification of remains presumably immersed for more than three weeks for which the only comparison sample was a 250-μL serum aliquot kept frozen in a laboratory. The analysis made it possible to identify a second victim as the daughter of the first.

KEYWORDS: forensic science, serum, DNA extraction, forensic, STR, filiation

Many serum markers are polymorphic and have been used in the past for the purpose of identification, filiation, and forensic studies. However, study of these markers requires a reasonable amount of serum, which was not available in the case reported here, and comparison was hampered due to the lapse of time between death and the corpse sampling. Moreover, the probability of finding two persons with the same serum markers is in the 10^{-1} to 10^{-2} range, whereas it is usually in the 10^{-6} to 10^{-9} range with ten STRs.

There are some reports in the literature related to extraction of DNA from serum (1). This is the basis for some prenatal diagnosis tests relying on the presence of fetal DNA in the mother's serum (2,3). The presence of DNA in serum is believed to result either from the presence of cell debris and/or from the presence of soluble DNA due to lysis of nucleated cells in the blood flow. The latter phenomenon would explain the presence of enzymatic activities in the serum as well. In this paper, we describe how we first established a suitable procedure for extracting DNA from serum and then used this procedure to elucidate a forensic case.

Case Report

The body of an unidentified, adult Asian female was found in the Seine River in the suburbs of Paris, France. Autopsy evidenced that nine knife wounds caused death. A few putrefactive lesions could be seen. Close to the supposed immersion spot, adult and children's clothes were found, as well as documents showing blood test results. It was possible to retrieve a 250-μL sample of serum in the laboratory where these blood tests were performed.

A five- to seven-year-old female Asian child was found at a dam downstream on the same river three months later. The autopsy revealed that the size of the child was consistent with the clothes found earlier, but the cause of death could not be established, and it was estimated that the rate of decay was consistent with three months of immersion.

Positive identification of the adult victim was made by comparison of the DNA extracted from the serum by means described below and showed the DNA to be that of the victim.

Comparing the DNAs of the adult and child revealed at least one identical allele in the eleven informative STRs, consistent with the hypothesis that the child was the daughter of the adult victim.

Materials and Methods

Extraction of Serum DNA (QIAamp Blood Kit, Qiagen)

In preliminary experiments, we used sera from 48 volunteers. Extractions were performed with the QIAmp blood kit. After a centrifugation step (15 min, 6000 g), the pellets were discarded and a 0.5-mL serum aliquot was digested in the protease (12.5 μL) and an AL buffer (500 μL) provided by the manufacturer. After incubation for 10 min at 70°C, 500 μL of ethanol was added. The solution was then loaded onto a QIAamp column (Qiagen). The washing and elution steps were strictly carried out as recommended by the manufacturer. For the forensic case serum, volumes were 0.1 mL for the serum, AL buffer, and ethanol.

Extraction of Tissue DNA (Qiagen Tissue DNA Extraction Kit)

DNA from a 25-mg sample of a heart (adult) and a 25-mg sample of unidentified tissue (child) was extracted strictly as recommended by the manufacturer. Briefly, the tissues were digested for 3 h in

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the proteinase K and ATL buffer, mixed with the AL buffer, then ethanol prior to loading onto the same columns as above. The washing and elution steps were performed with the buffers provided in the kit.

Microwave Treatment of Serum Prior to Amplification

An alternative procedure for preparation of DNA suitable for PCR was evaluated. Fifty microliters of volunteers' serum were irradiated in PCR tubes (0.2 mL) under oil for 5 min at 650 W. The tubes were next centrifuged 10 min at 13,000 × *g*, and the supernatants were used for the PCR.

DNA Extraction of the Control Whole Blood DNA (QIAamp Blood Kit, Qiagen)

The DNAs of the volunteers were extracted from the cell pellet of a blood sample. The procedure was carried out strictly as recommended by the manufacturer.

Genotyping

STR were amplified with the SGM plus and Profiler plus kits (Perkin-Elmer) and individual STR primers. For amplifications of TPOX, CSF1PO, F13, THO1, and FES/FPS, the PCR mix contained the relevant primers (Perkin-Elmer), 0.2 μ*M* each dNTP, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* Tris-HCl pH 8.3, and 1 IU Taq polymerase (Boerhinger Mannheim) in 25-μL reactions. For the SGM plus and Profiler plus, amplification parameters were as recommended except for the serum DNA, which was amplified under the optimal conditions (4) described by Gill (34 cycles). The other STRs were amplified for 30 cycles (tissue extracts) or 36 cycles (serum) in a Perkin Elmer 2400 thermocycler.

Results

Qiagen Extraction of Serum DNA

A total of 48 serum samples were prepared during three different experiments (range: 12 to 24 sera per extraction series) as described in the material and methods section.

In the initial experiments, Qiagen-extracted DNA sera were amplified with the primers for the F13A STR. The initial failure of STR amplification (30 cycles) led us to assess the amplification with 36 cycles. Under these conditions, 45 DNAs were amplified (90%), and three DNAs were unamplified at 36 cycles. The percentage of amplifiable DNA varied from 87 to 100% among DNA extraction series. None of the control water extracted with the Qiagen procedure showed any amplification.

Experimentally, we did not perform PCR with more cycles, because these conditions are already likely to detect very low copy numbers. Moreover, this would have increased the risk of detecting contaminating DNA or secondary DNA transfers.

Genotypes established from serum-extracted DNA were checked with F13 A genotypes determined from whole blood DNA. A total of 24 of the 48 relevant blood samples were extracted in separate procedures. No discrepancy was observed. These 24 blood cell DNAs were also amplified by means of the SGM plus kit and compared to the serum-extracted DNA amplified with the same kit, but with 34 cycles. Some allelic dropout was observed in the 24 relevant serum-extracted DNA. Despite the presence of allelic dropout or even failure of amplification for high-size alleles, this procedure was chosen for the forensic case study.

Microwave

Since we were aware of the possibility of DNA preparation in serum after microwave irradiation, we assessed this method. Initial attempts with 500 μL in 1.5-mL Eppendorf tubes were immediately stopped because of burst caps and spillover in the microwave. We were able to irradiate the serum in PCR tubes without cap bursting, providing sera were covered with PCR oil. However, the irradiated sera cannot be used directly for PCR due to the presence of protein coagulate. We had to centrifuge the tubes and use the supernatants. In a series of a total of 24 microwave-extracted DNAs, positive amplification of F13A was obtained in 13 sera (54%). Among the eleven remaining extracts, 40 cycles of amplification allowed for an extra six DNA typings (79% total). Thus, this procedure was aborted.

Forensic Case

The established routine procedure described above was used to extract the forensic serum sample. Each DNA extract was amplified at least three times, and Fig. 1 gives an example of representative amplification patterns. The probability of identity between the serum DNA and core DNA sample was 99.9983%. The probability of finding one individual with the serum alleles was 1 in 116,670,000,000. The maternity index was 99.998%.

However, dropouts were observed, even on the sample from the less-decayed body. These dropouts are likely to result from the degradation of the DNA. It is apparent from Table 1, which shows the results of the run presented in Fig. 1, that a decline occurred at Loci D21S11 or THO1 for the mother's core DNA. It is possible that better-quality DNA could have been obtained with other tissues, such as bone, but we did not investigate the possibility, since conclusions could be drawn from the available data.

TABLE 1—*STR data for the serum DNA and the DNA extracted from both victims. The underlined alleles are the alleles consistent with a mother-to-daughter relationship. The data presented here are those shown in Fig. 1 and represent one the three amplifications.*

Locus	Mother	Putative Child	Serum
F13A	3.2,–	3.2,–	3.2,–
AMG	X	X	X
D3S1358	15, 18	15,–	15, 18
VWA	16,–	16, 20	16,–
D16S539	9, 11	11,–	9, 11
D2S1338	–,–	18, 25	–,–
D8S1179	11, 15	11, 13	11, 15
D21S11	29, 30	30, 32.2	29,–
D18S51	–,–	17, 25	17,–
D19S433	13, 14	14,–	13, 14
THO1	6, 9	6, 7	6,–
FGA	23, 26	21, 26	23, 26
D5S818	11, 12	12,–	11,–
D13S317	9, 11	11, 12	–,–
D7S820	–,–	11, 12	11,–
CSF1PO	10, 12	10, 12	–,–
TPOX	8, 9	8, 9	8, 9
FES/FPS	11, 12	–,–	–,–

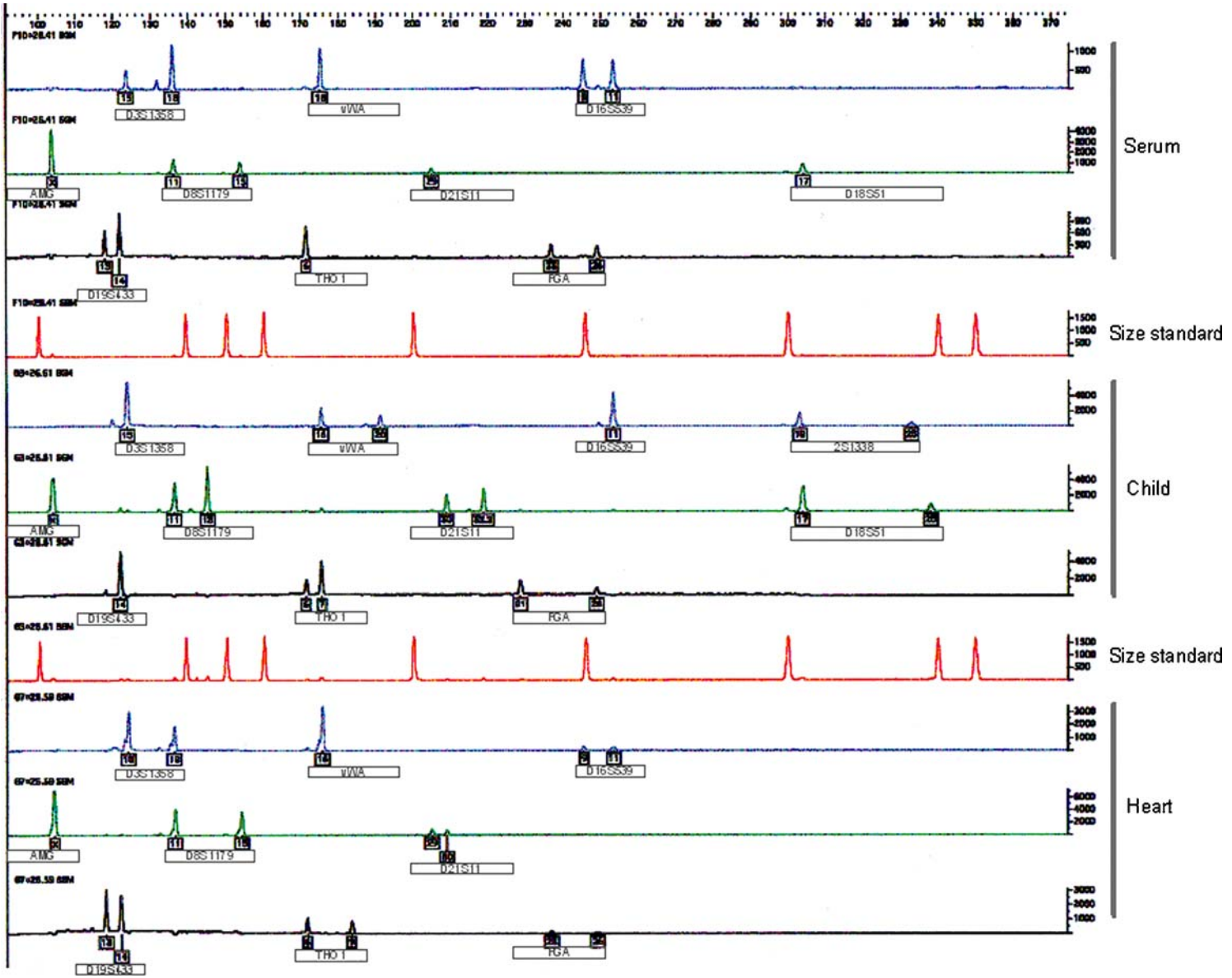

FIG. 1—*Electrophoregrams of the DNA amplified with the SGM plus kit. The data presented here are the ones given in Table 1 and are representative of one amplification out of the three. The top three lines (F10.26.41) show DNA extracted from the serum. The three middle lines (G3.26.61) indicate DNA extracted from the child's tissue, and the three bottom lines (G7.26.59) DNA taken from the mother's heart tissue.*

Discussion

Amplification of DNA extracted from serum was initially used for the detection of bacterial or viral sequences. However, interest has improved since the discovery of the presence of fetal DNA in the serum of pregnant women (5). This opened the route for noninvasive prenatal diagnosis, and numerous studies, reviewed in Refs 2 and 3, revealed that some prenatal diagnoses can be made providing the gene of interest is not present in the mother genome. Attempts to quantify the DNA in serum indicated that the amount of fetal DNA in maternal serum during the first trimester is in the 30 copies/mL range (6,7) and that the amount of maternal genomic copies is in the 1000 to 3000 copies/mL range. However, when dealing with forensic samples, feasibility is hampered by the nature of STR sequences. Because of Taq DNA polymerase slippage and low or absent proofreading activity, when dealing with very low copy numbers allelic dropouts, allele imbalance, or aberrant alleles are often observed even in the absence of contamination. It has been established by Gill (4) that for samples below 100 pg of DNA, successful genotyping can be achieved with 34 cycles for the SGM plus or Profiler plus kits. Moreover, a consensus emerged for not using more than 34 cycles. Thus, whereas it is possible to detect as low as a few copies of RhD or SRY DNA sequences in maternal serum by means of quantitative PCR (8), it is unclear if the amount of DNA in serum is suitable for a forensic identification. For example, Lo et al. were able to amplify DNA with as low as 10 μL of serum as a source of DNA (5). However, other protocols require 400 μL of serum as a starting material (8). We show here that the amount of DNA extracted with a method similar to the one described in Zhong et al. (8) leads to suitable STR amplification without breaking the consensual rule of 34 cycles when using the SGM plus or Profiler plus kits. The initial procedure found in the literature (1) was not used because of the severe drawback of biological sample spilling. In contrast, the simple Qiaamp blood procedure, which has been successfully used for prenatal diagnosis, appeared quite satisfactory. Serum DNA analysis also looks promising for the analysis of mutations in patients for which only serum was saved before DNA analysis was routinely available.

In the forensic case reported here, the DNA extraction was facilitated by the presence of a barely detectable pellet after centrifugation in favor of the presence of cell debris in the serum. We used these debris mixed with 100 μL of serum supernatant for DNA extraction and positive identification of a partially decomposed body. To our knowledge, it is the first successful attempt of DNA extraction from serum in a forensic case. The possibility of extracting DNA from serum should now be considered as an alternative to polymorphic serum markers, especially in cases like this one, where the decomposition status would not make reliable analysis possible. In the case of serum DNA amplifications of D2S1338, D13S317, CSF1PO, and FES/FPS, which failed at 36 cycles, we chose not to try with cycles to prevent detection of contaminating sequences. However, the amplified alleles made comparison and positive identification of the corpse possible.

An allelic dropout was observed for many STRs, and failure of amplification occurred for some others, probably because of lower yields of this particular PCR's reactions. Under this amplification procedure, allelic dropout does not necessarily involve the largest alleles. Thus, we did not conclude whether the presence of only one allele for the serum was due to homozygosity. In our experience, such a dropout is very common for degraded or low amounts of DNA, and, very often, increasing the number of cycles in the PCR does not allow for the amplification of the missing STR, but, rather, induces the appearance of extra, nonspecific peaks whose sizes may be confusing. We did not try to perform more cycles.

In conclusion, the method reported here appears suitable for extraction of DNA for serum, which makes identification and filiation studies possible in forensic cases. This might be used in genetic studies in medical practice for patients with only serum or plasma available, providing the occurrence of dropout is not an issue.

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