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TECHNICAL NOTE

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Successful DNA Typing of Urine Stains Using a DNA Purification Kit Following Dialfiltration

ABSTRACT: To evaluate the utility of DNA polymorphism typing of urine stains in forensic investigations, the amplifiable amount of DNA was estimated in 20 urine specimens obtained from 10 male and 10 female volunteers using a DNA purification kit following dialfiltration. DNA obtained from both urine and urine stains was amplified with the AmpflSTR® Profiler™ PCR Amplification Kit, and was analyzed by capillary electrophoresis using the Genetic Analyzer. The amount of male and female urine necessary for obtaining a complete DNA profile was 0.2 mL and 0.08 mL, respectively. When 0.2 mL of male urine were used to create urine stains, complete DNA profiles could be obtained from just some of the stains. However, when only 0.1 mL of female urine was used, complete profiles could be successfully obtained from all of the stains. DNA on bleached cotton remained amplifiable for 3–6 weeks. This method using a DNA purification kit following dialfiltration can be recommended for the genotyping of urine stains.

KEYWORDS: forensic science, urine stain, DNA typing, DNA purification kit

Forensic investigation of human urine stains is of great importance when attempting to ascertain the exact location of a crime and the precise manner of death. We have previously reported a method for identifying human urine stains utilizing high-performance liquid chromatographic (HPLC) analysis of 17-ketosteroid conjugates (1). Personal identification of urine stains is also indispensable if we are to specify that the urine was in fact excreted by a particular victim. Some attempts at the personal identification of urine and urine stains have been reported, such as the use of ABO blood typing and the typing of isozymes (2–4). There have also been several reports regarding the DNA typing of urine itself (5–8), but not of urine stains. DNA typing is currently the most valid method for the personal identification of human bodily fluid stains found at crime scenes. In our brief report (9), we previously indicated the possibility of procuring DNA profiles from urine stains, and although this was successful, it was with varying sensitivity. In this study, we decided to undertake the typing of urine stains using a DNA purification kit following dialfiltration. To evaluate the effectiveness of this procedure, an attempt was also made to estimate the amplifiable amount of DNA in urine.

Materials and Methods

Samples

Urine and oral mucous membrane samples were collected from 20 volunteers (10 males and 10 females). Some of the urine samples were soaked onto pieces of bleached cotton cloth in order to create urinary stains with a volume of 50, 100, 200 and 1,000 μL. Urinary DNA typing was independently confirmed by contrasting the profiles obtained from urine with those obtained from oral DNA typing.

DNA Extraction from Urine Samples

A urine sample (2 mL) was concentrated to 10–20 μL with the Centricon-100 device (Amicon Inc., Beverley, MA). DNA isolation was performed with a procedure for dried blood spots described in the pamphlet provided with the QiAamp DNA Microkit, as follows. One hundred and eighty microliters of buffer ATL and 20 μL of Protease K were added to the concentrated samples, and then incubated at 56°C for 1 h. Two hundred microliters of Buffer AL (containing carrier RNA) were added and the mixture was then incubated at 70°C for 10 min. An extract was applied onto a Microkit cartridge, and the cartridge was then washed, first with 500 μL of Buffer AW 1, and then with 500 μL of Buffer AW 2. Then the DNA was eluted with 25 μL of Buffer AE. Twenty microliters of the DNA solution were obtained from each of the samples. Buffer ATL, Protease K, Buffer AL, Buffer AW 1, Buffer AW 2 and Buffer AE were all provided with the kit.

DNA Extraction From Urine Stains and Oral Mucous Membrane

A piece of cloth containing a urine stain was cut into sections, and sections were steeped in 2 mL of PBS (pH 7.4) at room temperature for about 1 h to enable an extract to be obtained from the stain. The extract was then concentrated to 10–20 μL with the Centricon-100 device. DNA isolation was performed with a procedure for dried blood spots described in the pamphlet provided with the QiAamp DNA Minikit, as follows. One hundred and eighty

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microliters of Buffer ATL were added, and then the concentrated samples were incubated at 85°C for 10 min. Twenty microliters of Proteinase K were added and then the mixture was incubated at 56°C for 1 h. Two hundred microliters of buffer AL were added and then the mixture was incubated at 70°C for 10 min. Finally, 200 μL of ethanol were added. An extract was applied onto a Minikit cartridge, and the cartridge was then washed, first with 500 μL of Buffer AW 1, and then with 500 μL of Buffer AW 2. Then, the DNA was eluted with 400 μL of Buffer AE. Precipitation of DNA was carried out with ethanol. The DNA precipitation was dried and resuspended in 10 μL of TE buffer. Ten microliters of the DNA solution were obtained from each of the samples, and then the entire amount of the solution underwent PCR amplification.

DNA extraction from oral mucous membrane was also performed using the procedure for buccal swab as described in the pamphlet provided with the QiAamp DNA Minikit.

Amplification and Electrophoresis of DNA

All materials recovered from the urine samples were amplified with the AmpflSTR® Profiler™ PCR Amplification Kit (Applied Biosystems, Foster city, CA) on a GeneAmp System 9700 thermal cycler (Applied Biosystems, Foster city, CA). PCR amplification was performed in a final volume of 25 μL, composed of 10 μL of the PCR reaction mix, 5 μL of the primer set, 0.5 μL of AmpliTaq Gold DNA polymerase, and 10 μL of the concentrated sample. The conditions for PCR amplification were as recommended by the manufacturer.

Capillary electrophoresis was carried out using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster city, CA). 1.5 μL of amplified products and 0.5 μL of internal size standard (GeneScan-350 ROX, Applied Biosystems, Foster city, CA) were added to 25 μL of deionized formamide. After denaturation, the PCR products were run as described in the protocol for the AmpflSTR® Profiler™ Kit. The length of amplified fragments was established from the internal run standard by the Southern Local method using GeneScan Analysis 3.1.2 software (Applied Biosystems, Foster city, CA). Allele typing was carried out by Genotyper 2.5 software (Applied Biosystems, Foster city, CA).

Estimation of the Amplifiable Amount of Urine

The DNA obtained from 20 urine samples (10 males and 10 females) was amplified with the AmpflSTR® Profiler™ Kit as set out in Fig. 1. Eight amplified fragments were obtained from each of the urine samples. Accordingly, allele typing was carried out on one hundred and sixty fragments in order to estimate the amplifiable amount of urine.

DNA Typing of Urine Stains

DNA extractions and allele typing were carried out on the urine stains created from 50, 100, and 200 μL of urine provided by 10 volunteers (5 males and 5 females). At between 1 week and 6 weeks, one quarter of the urine stains created with 1000 μL of urine obtained from 4 volunteers (2 males and 2 females) underwent DNA extraction and allele typing.

Results

Table 1 shows the results obtained from electropherograms of the PCR products from various amounts of DNA solution obtained from urine. One microliter of the original solution represented 10 μL of the diluted solution for PCR amplification. Moreover, 1 μL of the original solution represented 200 μL of urine since the DNA was extracted from 2 mL of urine. As for the male samples, although DNA profiles were obtained from seven samples, it was not possible to obtain profiles from the other three samples. Partial profiles, with some loci amplified, were obtained from only 4 μL of diluted solution in two specimens. Complete profiles, with all loci amplified, and a partial profile were obtained from extracts of 1–2 μL of original solutions in five and one specimens, respectively. On the other hand, with regard to the female samples, complete profiles were obtained from extracts using less than 8 μL of diluted solution, and in the case of some samples, it was possible to obtain complete profiles using only 1 μL of diluted solution. In all cases, the DNA profiles obtained from the urine samples were identical to the profiles obtained from the oral mucous membrane.

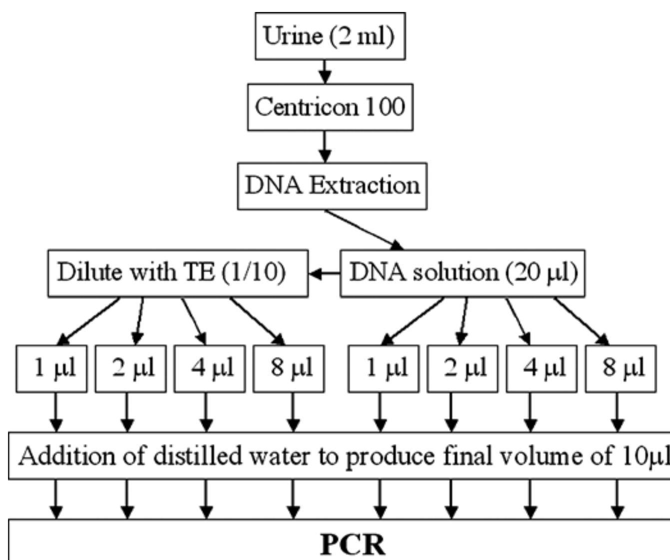

FIG. 1—*Procedure for estimation of the amplifiable amount of urine needed for PCR.*

An example of an electropherogram of PCR products from a urine stain created from a male sample is shown in Fig. 2. An example of an electropherogram of PCR products from a urine stain created from a female sample is shown in Fig. 3.

The results of DNA typing from urine stains created using only a small amount of urine are shown in Table 2. From the male samples, it was possible to obtain profiles from three out of the five samples, and accordingly, it would appear that in the case of male samples, the urine stains needed to have been created using samples comprising 100–200 μL of urine in order for profiles to be successfully obtained. However in the case of female samples, all the urine stains created from only 100 μL of urine provided complete profiles, with either complete or partial profiles being provided even by the 50 μL urine samples. The results of DNA typing using aged urine stains are shown in Table 3. Complete profiles could be obtained from all the urine stains, which were 1–3 weeks old. However, although profiles were not obtained from male urine stains, which were 4–6 weeks old, it was possible to obtain profiles from all of the 4-6-week-old female urine stains.

Discussion

Regarding the genotyping of urine, there have been some reports describing the DNA typing of pellets of cellular material from

TABLE 1—*The results of electropherograms of PCR products for different amounts of DNA solution obtained from urine. Since off-scale data were obtained from some samples, capillary electrophoresis was again carried out on the diluted fragments.*

		Diluted Solution (1/10)				Original Solution (1)			
Amount for PCR		1 μL	2 μL	4 μL	8 μL	1 μL	2 μL	4 μL	8 μL
Converted Quantity of Urine		10 μL	20 μL	40 μL	80 μL	100 μL	200 μL	400 μL	800 μL
Male	1	−	−	±	±	+	+	+	+
	2	−	−	−	±	±	+	+	+
	3	−	−	−	−	−	−	−	±
	4	−	−	−	−	−	−	−	−
	5	−	−	−	−	−	±	+	+
	6	−	−	−	−	−	−	−	−
	7	−	−	−	−	−	−	±	+
	8	−	−	−	±	±	+	+	+
	9	−	−	−	±	+	+	+	+
	10	−	−	±	+	+	+	+	+
Female	1	+	+	+	+	+	+	+	+
	2	−	−	±	+	+	+	+	+
	3	±	±	+	+	+	+	+	+
	4	−	±	±	+	+	+	+	+
	5	−	−	±	+	+	+	+	+
	6	±	±	+	+	+	+	+	+
	7	+	+	+	+	+	+	+	+
	8	−	±	±	+	+	+	+	+
	9	±	±	+	+	+	+	+	+
	10	−	±	+	+	+	+	+	+

− : Negative profile, ±: Partial profile, +: Complete profile.

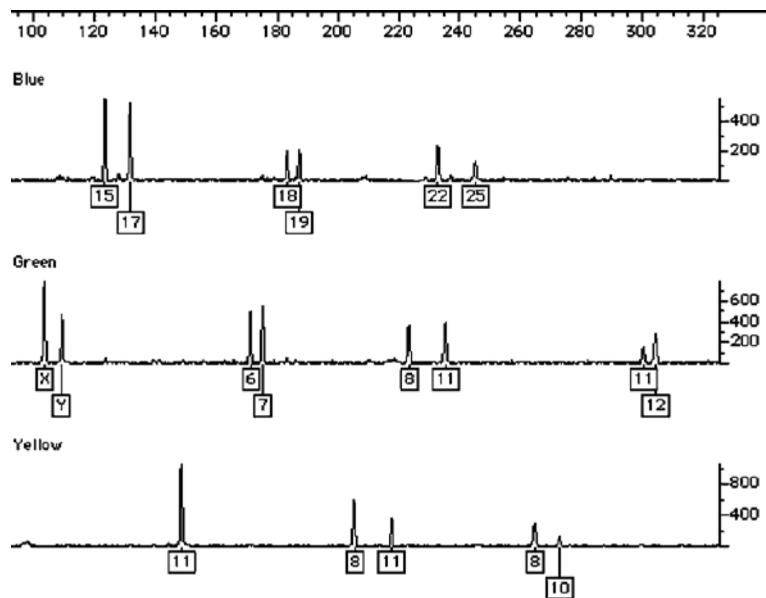

FIG. 2—*An electropherogram of the STR results obtained from a urine stain—Urine stain: 3-week-old urine stain created from male urine (200 μL of urine soaked on pieces of bleached cotton). The vertical scale is the fluorescence intensity in relative fluorescence units (RFU), while the horizontal scale is the length of the amplified fragments in nucleotide bases. The numbers under the peaks refer to the allelic designation at the individual loci. The genetic loci indicated are from left to right, D3S1358, vWA and FGA (top: Blue); amelogenin, TH01, TPOX and CSF1PO (middle: Green); and D5S818, D13S317 and D7S820 (bottom: Yellow).*

centrifuged urine (5–8). Purification of samples by dialfiltration usually facilitates the collection of both cells and the free high molecular DNA which is released from the cells, however, Vu NT et al. reported that in their experiment, they were not able to obtain good results regarding DNA extraction following dialfiltration of urine (6). Moreover we have not been able to obtain good results of genotyping from urine using phenol-chloroform extraction following dialfiltration. One reason for our success in the genotyping of urine following purification by dialfiltration using the Centricon-100 device is due to the DNA purification kit used in this study, since QiAamp purification kits have provided good results regarding DNA isolation from forensic samples in the past (10–11). Although we used two different purification kits for urine and urine stains (QiAamp DNA Microkit and QiAamp DNA Minikit, respectively), we feel that it is necessary to carry out further studies in order to ascertain which DNA purification kits are the most reliable for urine and urine stains. Determination of the amount of DNA in a sample is essential for most PCR-based assays (12). We

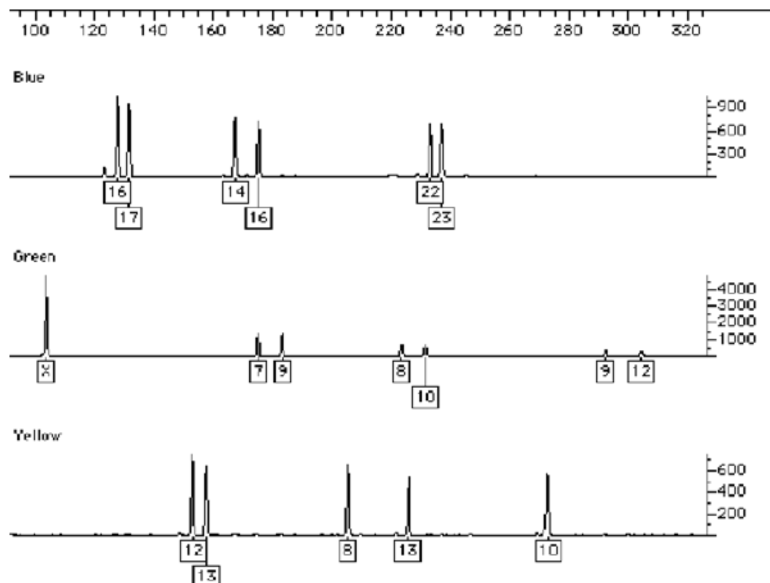

FIG. 3—*An electropherogram of PCR products from a urine stain—Urine stain: 2-week-old urine stain created from female urine (50 μL of urine soaked on pieces of bleached cotton.) The vertical scale is the fluorescence intensity in relative fluorescence units (RFU), while the horizontal scale is the length of the amplified fragments in nucleotide bases. The numbers under the peaks refer to the allelic designation at the individual loci. The genetic loci indicated are from left to right, D3S1358, vWA and FGA (top: Blue); amelogenin, TH01, TPOX and CSF1PO (middle: Green); and D5S818, D13S317 and D7S820 (bottom: Yellow).*

TABLE 2—*DNA typing from urine stains created from a small amount of urine.*

	Sample	50 μL	100 μL	200 μL
Male	1	−	±	+
	2	−	±	+
	3	−	−	−
	4	−	−	−
	5	−	−	±
Female	1	+	+	+
	2	−	+	+
	3	±	+	+
	4	+	+	+
	5	+	+	+

− : Negative profile, ±: Partial profile, +: Complete profile.

TABLE 3—*DNA typing from aged urine stains created from samples comprising 250 μL of urine.*

	Sample	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks	6 Weeks
Male	1	+	+	+	−	−	−
	2	+	+	+	−	−	−
Female	1	+	+	+	+	+	+
	2	+	+	+	+	+	+

− : Negative profile, +: Complete profile.

tried to determine the amounts of DNA in urine were quantified at 260 nm using a UV spectrophotometer, however it was impossible to determine this because the amounts of DNA in the urine samples were too small. So, we attempted to estimate the amplifiable amount of urine, and based on the results presented in Table 1, it was possible to estimate this. In the case of males, 100–200 μL of urine were needed in order to obtain the profile for genotyping. On the other hand, in the case of females, it was possible to obtain a complete profile from only 40–80 μl of urine. Accordingly, it is easier to obtain a complete profile from female samples than from male samples. Prinz et al. reported similar results (13), suggesting that urine from females contained more epithelial cells than that from males and that this could explain the gender-specific difference in DNA yields. Our experiments of DNA typing from urine stains were carried out after consideration of the results of DNA typing from urine, and similar results were observed. It thus follows that when obtaining profiles from male urine stains, a larger amount of urine is necessary than is the case with female urine stains. Moreover, in the case of female urine samples, it was possible to obtain profiles for the genotyping of aged urine stains, even after a few weeks had passed. This can also be explained by the reasoning of Prinz et al. (13).

To conclude, it was possible to carry out DNA typing from urine stains obtained from small amounts of urine samples, and even from urine stains that were about one month old. We therefore think that it is appropriate when carrying out a forensic investigation to carry out DNA typing from urine stains found at a crime scene. There is a difference between male and female urine samples regarding the quantity of DNA available, however, since in most cases there is no information regarding gender when urine is collected from a crime scene, it should always be collected from the largest urine stains that are available. When carrying out DNA typing from a urine stain found at a crime scene, it would be helpful if a relative cell count in the sample before isolating the DNA, as well as a determination of the amount of DNA in the sample using either a QuantiBlot® Human DNA Quantitation kit (Applied Biosystems, Branchburg, New Jersey) or some other method could both be carried out.

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