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TECHNICAL NOTE

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A Simple Method of DNA Extraction and STR Typing from Urine Samples Using a Commercially Available DNA/RNA Extraction Kit*

ABSTRACT: We devised a simple DNA extraction procedure suitable for STR typing of urine sample. Use of a commercially available DNA/RNA extraction kit equipped with a silica-gel-based membrane made it possible to omit the recovery of urinary nucleated cells by sedimentation before the extraction. Successful genotyping of the TH01, HumTPO and multiplex STRs was achieved using aliquots of urine as small as 100 μL. Furthermore, application of this DNA extraction procedure to frozen urine samples provided STR allele results comparable to results obtained from fresh samples. Therefore, this extraction procedure is considered to be effective for STR typing of urine samples in both the frozen and aqueous state. Furthermore, addition of sodium azide to fresh urine samples prolonged their storage duration even at room temperature.

KEYWORDS: forensic science, DNA typing, DNA extraction, storage condition, short tandem repeat typing, TH01, HumTPO, multiplex, urine

Human urine is frequently sampled for toxicological analysis in doping and drug screening tests, and the origin of the samples may need to be verified. In this context, there is a need to develop forensic methods for individualization of urine samples using various genetic markers. In addition to a few serological genetic markers such as ABO blood group, at least ten kinds of biochemical genetic markers present in urine have been newly discovered and characterized in our laboratory (1). The presence of nucleated cells in urine, even in small amounts, facilitates PCR-based DNA typing from the corresponding samples (2–6). However, in all the DNA extraction procedures from urine samples employed previously, a step for recovery of urinary cells by sedimentation has been indispensable. Furthermore, freeze-thawing of urine samples results in destruction of any urinary tract epithelial cells present, thus limiting the possible source of genomic DNA (7). These problems may hinder the long-term storage of urine samples in the frozen state. Also, the amount of DNA extractable from urine samples depends on the DNA extraction method employed (8). Commercially available DNA/RNA extraction kits using various chromatography resins such as silica-gel and anion-exchange resin have been more popular for DNA extraction than the conventional organic and Chelex extraction methods, and their use was expected to overcome the defects inherent in the previous DNA extraction and storage of urine samples.

Here we describe a simple DNA extraction procedure suitable for STR typing of urine samples, using a commercial DNA/RNA extraction kit that avoids the step of having to recover urinary cells, and can be applied to samples stored under a variety of conditions.

Materials and Methods

Biological Samples

Urine samples, together with blood samples, were collected from healthy Japanese volunteers including laboratory workers and students, from whom written informed consent had been obtained. After thorough mixing by gentle inversion, each freshly taken urine sample was divided into four different portions in appropriate tubes. To each of these aliquots, sodium azide, EDTA, or both, at a final concentration of 0.1% (w/v) and 40 mM, respectively, were added separately. The remaining sample was left untreated. These different samples derived from the same volunteers were kept at room temperature, 4°C, −30°C or −80°C for one day and 1–5 weeks.

DNA Extraction from Urine

DNA was isolated from the urine specimens using a commercially available DNA/RNA extraction kit, QIAamp Viral RNA Mini Kit (QIAGEN K.K., Tokyo, Japan). After thorough agitation, 140-μL aliquots of each were processed for DNA extraction

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mainly according to the manufacturer's instructions, except that neither concentration of the urine samples using the centrifugal microconcentrators recommended by the manufacturer, nor sedimentation (8) prior to DNA extraction, was performed. Briefly, aliquots of urine samples were mixed with 560 μL of the lysis buffer supplied in the kit and 560 μL of ethanol, and then applied to a spin-column equipped with a silica-gel-based membrane. After washing the column, urinary DNA was finally recovered in 45 μL of 10 mM Tris-HCl buffer (pH 8.0). Five-microliter aliquots of the DNA preparation were used for the subsequent PCR amplifications. These experiments were carried out in triplicate per preservation condition. Also, corresponding DNA samples were prepared from blood buffy coats from the same volunteers using a QIAamp DNA Blood Kit (QIAGEN K.K.). All the DNA samples obtained were stored at −80°C until typed.

STR Typings

PCR amplification was performed with a TH01 STR system using a *GenePrint*® Fluorescent Monoplex STR system (Promega K.K., Tokyo, Japan) and with multiplex STR system using a PowerPlex® 16 system (Promega K.K.), followed by determination of the genotypes using a Genetic Analyzer (model 310, Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Also, the HumTPO and TH01 STR systems were examined by discontinuous, horizontal polyacrylamide gel electrophoresis and silver staining according to the method described previously (9–11). Since carrier RNA was added to the lysis buffer supplied in the kit in order to improve the recovery of DNA which is present in low amounts present in urine, the concentration of the urinary DNA recovered could not be determined. Instead, the success or failure of these STR typings of the corresponding DNA samples was examined to evaluate the intactness and suitability of the urinary DNA recovered as a template for the STR typings.

Results and Discussion

Since urine often contains very low numbers of nucleated cells such as epithelial cells and leukocytes as a source of urinary DNA, concentration of urine samples to recover the corresponding cells by sedimentation (2,3,7) or diafiltration (8) prior to DNA extraction has been indispensable for DNA typing. However, when 140-μL aliquots of fresh urine samples from volunteers were subjected directly to DNA extraction using a commercial DNA/RNA extraction kit without such a concentration step, we were able to carry out successful typing of the TH01, HumTPO and multiplex STRs from each sample of urine DNA. These genotypes determined from a urine specimen from each volunteer matched perfectly those from the corresponding blood sample. Since the lysis buffer supplied in the kit we used inactivates the numerous unidentified PCR inhibitors present in urine (12), the step for elimination of the inhibitors to improve the PCR amplification efficiency was not necessary. Therefore, the simple DNA extraction procedure without concentration of urinary cells using the commercial extraction kit was employed for STR typing throughout this study. The amount of fresh urine required for these STR typings was estimated to be as little as 100 μL, irrespectively of gender. Furthermore, the amounts of urine required for TH01 typing using fluorescence detection were similar to those for silver staining, indicating that these amounts may be sufficient for STR typing by either of the methods (13). Also, another commercial extraction kit, the QIAamp DNA Blood Kit, was similarly effective for DNA extraction and STR typing of urine samples.

In order to examine the effects of storage temperature, aging and presence of preservatives on urinary DNA extraction and typing efficiency, a fresh urine sample taken from a male volunteer was used to ensure uniformity of the DNA concentrations obtained (Table 1). Correct typing was performed from a urine sample kept at 4°C for three weeks, but not for only one week at room temperature. Freezing and thawing of urine samples may result in cell lysis, with concomitant release of nuclear DNA into the urine matrix and in this state it has been postulated that DNA is subjected to hydrolysis by various endogenous nucleolytic enzymes present in the urine (14–16). In the previous DNA extraction methods that included a step for concentration of urinary cells by sedimentation, frozen urine specimens were not typable as effectively as fresh ones (7,8). However, our DNA extraction method, in which DNA was directly recovered from aliquots of frozen and thawed urine without such a concentration step, permitted frozen urine samples to be a suitable source of template DNA for STR typing that was not inferior to DNA from fresh samples. In fact, all the frozen urine samples tested gave typable results. Fresh urine samples collected from other volunteers (two males and one female) gave the same results on the effects of storage temperature, aging and presence of preservatives on urinary DNA extraction and typing efficiency as that from the former volunteer. Also, urine samples subjected to several freeze-thaw cycles showed no deleterious effects in terms of STR typing results.

We have used sodium azide as a preservative of urine samples for biochemical marker typing and obtained good results (1). Adding sodium azide to urine samples at a final concentration of

TABLE 1—*The typing results for the TH01, HumTPO and multiplex STR systems from urine samples kept under different conditions.* +, typable; −, not typable.*

	Room Temperature				4ºC				−30ºC or −80ºC		
	None	NaN_3	EDTA	NaN_3+ EDTA	None	NaN_3	EDTA	NaN_3+ EDTA	None	NaN_3	NaN_3+ EDTA
Storage period											
1 day	+	+	+	+	+	+	+	+	+	+	+
1 week	−	+	−	+	+	+	+	+	+	+	+
2 weeks	−	+	−	+	+	+	+	+	+	+	+
3 weeks	−	−	−	−	+	+	+	+	+	+	+
4 weeks	−	−	−	−	−	+	−	+	+	+	+
5 weeks	−	−	−	−	−	+	−	+	+	+	+

* Urine sample (140 μL) collected from a male volunteer and kept under various conditions was subjected to the DNA extraction as described in the text, followed by typing of TH01, HumTPO and multiplex STRs.

0.1% (w/v) gave good typing results for urine samples stored at both room temperature and 4°C: all the urine samples ($n = 5$) with added sodium azide kept for five weeks at the latter temperature were typable. However, addition of EDTA, which has been reported to increase the stability of DNA over long storage periods, especially at low temperature (17), did not further extend the storage duration of urine samples kept at both temperatures. Therefore, we recommend that sodium azide is added to fresh urine samples as a preservative to allow long-term storage in an unfrozen state. Also, all the frozen urine samples tested ($n = 5$), which were kept at −30°C and −80°C for up to at least three months after addition of sodium azide, were typable without difficulty.

Overall, simple DNA extraction, using a commercial DNA/RNA extraction kit equipped with a silica-gel-based membrane without a step for concentration of urinary cells, allows urine samples to be kept in both a frozen or aqueous state and is more applicable for STR typing of urine samples. Also, for long-term storage of urine samples at room temperature and 4°C, addition of sodium azide to freshly taken urine samples, is effective.

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