Journal of Clinical Laboratory Analysis 12:88–91 (1998)

DNA Extraction From Human Urinary Sediment

M. Yokota,* N. Tatsumi, I. Tsuda, T. Takubo, and M. Hiyoshi

Department of Clinical and Laboratory Medicine, Osaka City University Medical School, Osaka, Japan

DNA was extracted from urinary sediments and was sufficient for polymerase chain reaction (PCR) and enzymatic analysis, even if DNA from microorganisms coexisted. From urine samples, the yield of DNA ranged from trace levels to 20 μg per 10 mL urine. When urinary sediment was stored in ethanol, DNA remained stable for 2 weeks or more. Individual identification and sex determination could easily be performed using either fresh or ethanol-fixed urine. In conclusion, urine can be used as a source for PCR-based investigations and genetic studies. J. Clin. Lab. Anal. 12:88–91, 1998. © 1998 Wiley-Liss, Inc.

Key words: urine; HLA typing; sex determination; PCR

INTRODUCTION

Urine is examined daily in the clinical laboratory as part of routine testing and predictably contains erythrocytes, leukocytes, and squamous cells, even in normal subjects (1). With the exception of sperm in specimens from women, all nucleated cells present contain the subject's DNA. In the clinical laboratory, urine usually is discarded after microscopic assessment. We assessed whether urine additionally could be used for DNA analyses.

MATERIALS AND METHODS

Urine was obtained from healthy volunteers ranging in age from 12–35 years (16 males and 5 females) and from 125 patients of each gender, ranging in age from 5–84 years, who were undergoing medical consultation at the university hospital of Osaka City University Medical School. (The study was approved by the Institutional Review Board of the university.) Fresh urine (10 mL) were centrifuged at 1,500 rpm for 5 min, after which 9.8 mL of the supernatant were discarded. The sediments were resuspended in the remaining 0.2 mL, and two drops of dye solution, modified stains of Sternheimer and Malbin, were added (Wako Pure Chemicals Co. Osaka, Japan). Semiquantitative assessment of the number of elements per high power microscopic field was performed. Another 10 mL aliquot, from urine stored at 4°C for 12–20 hr, was centrifuged at 3,000 rpm for 10 min. The sediment thus obtained was mixed with 1 mL of TNE buffer (10 mM of Tris-HCl at pH 8.0, 1 mM of Na_2 EDTA, and 100 mM of NaCl) and re-centrifuged.

DNA Extraction

Urine sediment, obtained as described above, was collected into a polypropylene tube (1.5 mL), and 400 μL of lysing solution was added (TNE buffer at pH 8.0 containing 1% sodium dodecyl sulfate and 100 μg/mL proteinase K from Wako). The mixture was incubated for 4 hr at 55°C, extracted once with TE phenol, representing phenol saturated with TE buffer (10 mM of Tris-HCl and 1 mM of Na_2 EDTA, pH 8.0), twice with TE phenol:chloroform: isoamyl alcohol (25:24:1, by volume), and once with chloroform:isoamyl alcohol (24:1, by volume), retaining the upper aqueous phase at each step. Sodium acetate was added to a final concentration of 0.3 M, and the DNA was precipitated with two volumes of chilled absolute ethanol (EtOH) at –20°C overnight, followed by centrifugation at 15,000 rpm for 15 min. After washing with cold 70% EtOH, the pellets were dried and resupended in 30 μL of TE buffer at 37°C. In the event that large amounts of DNA strands were detected, the pellets were resuspended in 600 μL of TE buffer. Extracted DNA was quantified by spectrophotometric analysis at 260 nm (OD_{260}), using a UV2200 spectrophotometer (Shimadzu, Kyoto, Japan) and/or estimated by gel electrophoresis as follows. Five μL of each specimen were loaded on a 1% agarose gel (Wako) in a minigel apparatus (Mupid, Cosmo Bio, Tokyo, Japan) and subjected to electrophoresis. DNA was visualized by ethidium bromide staining and photographed under ultraviolet light. If the gel was overloaded, electrophoresis was repeated at a higher dilution. DNA quantity was estimated by comparison to 10, 20, and 30 ng DNA standards.

*Correspondence to: Masaharu Yokota, Department of Clinical and Laboratory Medicine, Osaka City University Medical School, Asahimachi, Abeno, Osaka, 545 Japan.

Received 21 January 1997; Accepted 10 July 1997

PCR Amplification of HLA-DQA1 Gene and Human X-Y Homologous Region in X and Y Chromosomes

Amplification of the HLA-DQA1 locus was performed using the GH26 and GH27 primers (2) (Perkin-Elmer Cetus Instruments, Norwalk, CT), and amplification of the X-Y homologous region was performed using X-Y homologous primers (3), SXY-1001 and SXY-1002 (Maxim Biotech, San Francisco, CA). The PCR reaction mixture (50 μL) included 10 μL of extracted DNA sample (or < 400 ng of DNA), 200 μM of each deoxynucleotide triphosphate (dATP, dCTP, dGTP, and dTTP; Takara, Kyoto, Japan), 1.25 units of Taq DNA polymerase (Takara), 5 μL of 10x reaction buffer, and 10 pmol of each HLA-DQA1 primer or 25 pmol of each X-Y homologous primer. Samples were heated at 94°C for 5 min in the first round of denaturation. Samples were then subjected to 32 cycles of PCR consisting of 1 min at 94°C, 1 min at 62°C, and 2 min at 72°C for HLA-DQA1 and 1 min at 94°C, 1 min at 52°C, and 2 min at 72°C for the X-Y homologous region. Cycling was performed by automated DNA Thermal Cycler (GeneAmp PCR System 9600, Perkin Elmer Cetus). After the last cycle, the samples were incubated for an additional 5 min at 72°C. Amplified DNAs that should have resulted in a 242 bp product for the HLA-DQA1 gene and 977 and 788 bp products for the X-Y homologous region, respectively, were checked on a 3% (w/v) NuSieve GTG agarose gel (Takara) as described previously.

Availability of Extracted DNA to Endonucleases

The availability of extracted DNA for endonuclease digestion was checked using three restriction endonucleases in representative HLA typing sets, specifically HaeIII, DdeI, and RsaI for the HLA-DQA1 gene (4). For the X-Y homologous region, amplified DNAs from the X chromosome were digested with TaqI, whereas the fragment from the Y chromosome was digested with HpaII. Restriction fragments were detected by staining with ethidium bromide following electrophoresis as described above.

DNA Extraction From Urine Fixed With Absolute Ethanol

Urine samples in which a large number of leukocytes and squamous cells were detected were separated into 16 tubes with 10 mL of urine each. In four tubes, DNA in urine was extracted immediately as described above (Group A). In addition, 5 mL of absolute ethanol (EtOH) were added to the sediments from the remaining 12 tubes, which then were divided into three groups for storage for 2 weeks at 4°C (Group B), 25°C (Group C), or 30°C (Group D). Samples from these three groups were centrifuged and washed once with 1 mL of TNE buffer. DNA then was extracted as described above. Extracted DNA was resuspended in 600 μL of TE buffer, and quantity was determined by optical density and gel electrophoresis. Purity was estimated by assessing the ratio of absorbance (OD_{260}/OD_{280}).

Statistics

Student's t-test was used to determine significant differences between groups.

RESULTS

Relationships Between Cell Counts in Urinary Sediment and Amount of Extracted DNA

Trace amounts, < 30 ng of DNA, were extracted from 10 mL of urine obtained from healthy subjects (N = 21), even those specimens in which no cells were found in the urinary sediment. From urine samples of 250 patients, DNA yields ranged from trace levels to 20 μg (Fig. 1).

Samples with 5–20 epithelial cells and/or leukocytes in one high power microscopic field (x400 magnification) yielded ~200–1,000 ng of DNA. Samples with > 20 epithelial cells and/or leukocytes per high power field yielded > 1 μg of DNA (Fig. 1). Relatively high levels of DNA were extracted from urine containing microorganisms such as bacteria, yeasts, or *Trichomonas vaginalis*, and the extracted DNA was thought to contain DNA derived from these microorganisms. But the extracted DNA was useful for PCR amplification for HLA typing or sex determination based on the X-Y homologous region.

About 80–95% of observed leukocytes were neutrophils. In some samples, a variety of salt crystals and bilirubin, as well as erythrocytes, were found in the sediments, but no interference was observed in the phenol-chloroform DNA extraction or in PCR amplification. Additionally, the presence

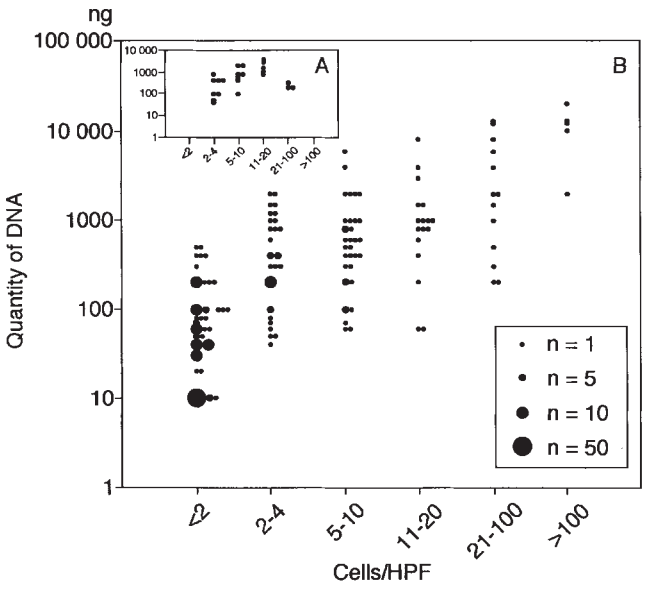

Fig. 1. DNA recovery vs. total cells in urinary sediments. X axis in **A** is the count of epithelial cells and that of **B** is epithelial cells and leukocytes. HPF, high-power field (×400).

of mucoid and crystals that grew during storage did not interfere with DNA extraction. Sufficient DNA for PCR analysis was obtained from 10 mL of urine in all cases (n = 271), except for the one male in whom no cellular components were detected microscopically in the sediment.

Determination of Electrophoretic Patterns of Extracted DNA

High molecular weight DNAs with a distinct or relatively broad band were visible in almost all samples. Degraded DNA was detected in a few specimens; clinical circumstances were examined in these cases. All extraction was started within 20 hr of random single-voiding collections or from 24-hr urine collections. Degradation usually was not observed after 24 hr of storage, but DNA degradation patterns were exhibited in some specimens from patients with cancer undergoing chemotherapy.

PCR Amplification and HLA-DQA1 Genotyping Using Extracted DNA

HLA-DQA1 genes could be amplified from all extracted DNAs. The genotypes of the HLA-DQA1 genes could be identified unambiguously (Table 1). The amplified 242 bp DNA product was digested with three restriction endonucleases; HaeIII, DdeI, and RsaI for HLA-DQA1 genotyping (Fig. 2).

Sex Determination After PCR Amplification of the X-Y Homologous Gene Amelogenin

Although the availability of amplified DNAs could be demonstrated by HLA-DQA1 typing, the DNAs were also assessed in another manner. Amplified bands of 977 bp on the X chromosome and 788 bp on the Y chromosome could be clearly sparated in all samples and were digested with TaqI and HpaII, respectively (Fig. 3). In all cases the determined sex corresponded with the sex noted in the medical records.

TABLE 1. Distribution of HLA-DQA1 Genotypes Assessed in Urine Samples

HLA-DQA1 subtypes	Number of specimens	Frequency (%)
0101,2/0101,2	32	11.8
0101,2/0103	14	5.2
0101,2/0301	64	23.7
0101,2/04,501	4	1.5
0101,2/0601	2	0.7
0103/010	14	5.2
0103/0301	43	15.9
0103/04,501	2	0.7
0103/0601	1	0.4
0201/0301	2	0.7
0301/0301	72	26.7
0301/04,501	11	4.1
0301/0601	6	2.2
04, 501/04,501	1	0.4
04, 501/0601	1	0.4
0601/0601	1	0.4
others	0	0
Total	270	100

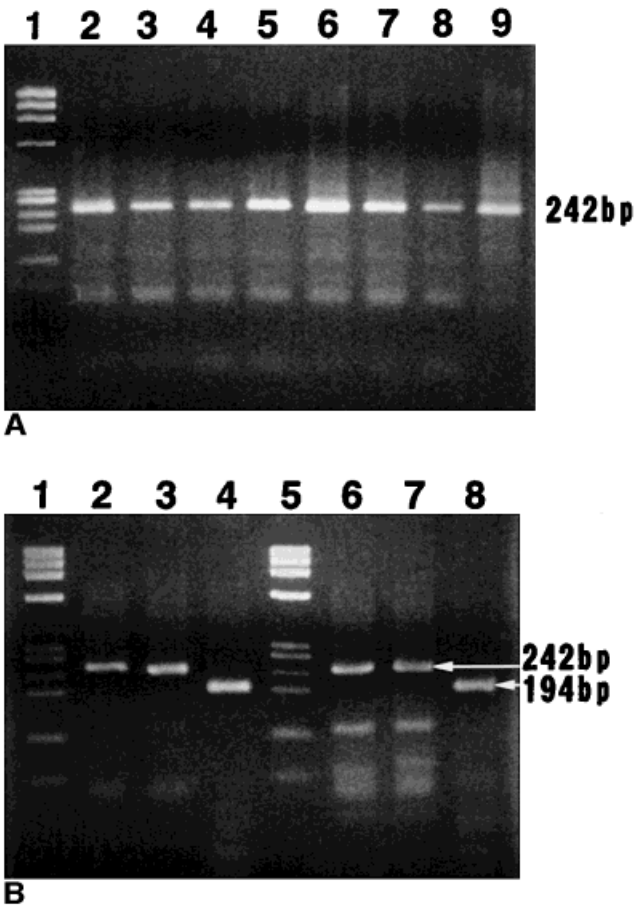

Fig. 2. Amplification of the HLA-DQA1 gene. **A.** Results of amplification with GH 26 and 27 primers from DNA extracted from urine (2–8) are shown. 1, ΦX174/HaeIII; 9, PCR positive control. **B.** Polymorphic restriction fragment patterns of PCR products. ΦX174/HaeIII (lanes 1 and 5), HaeIII digestion (lanes 2 and 6); DdeI digestion (lanes 3 and 7); RsaI digestion (lanes 4 and 8); HLA subtypes 0301/0301 (lanes 2–4) and 0101,2/0301 are shown (lanes 6–8).

Effect of Storage on Urine Fixed With EtOH

The above experiments were performed using urine specimens that were either freshly collected or stored for 24 hr. We also were interested in determining whether fixed and long-term-stored urine could be used for such typing.

The amounts of DNA extracted from urine in Group A (fresh), B (stored for 2 weeks at 4°C), C (stored for 2 weeks at 25°C), and D (stored for 2 weeks at 30°C) were 12.9 ± 0.25, 13.4 ± 0.39, 13.0 ± 0.08, and 13.2 ± 0.68 (mean ± SD for four determinations), respectively. The

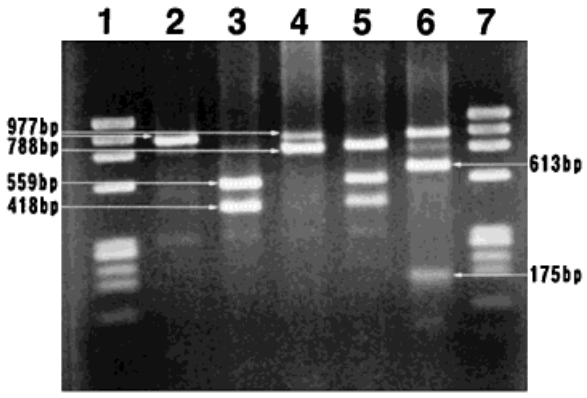

Fig. 3. Amplification of the X-Y homologous gene amelogenin. ΦX174 digested with HaeIII was used for size references (lanes 1, 7). An amelogenin gene fragment from a female and male were amplified (lanes 2 and 4, respectively) and digestion with TaqI (lanes 3 and 5, respectively). An amplified fragment from a male was digested with HpaII (lane 6).

ratio of absorbance (OD_{260}/OD_{280}) of all samples was ≥ 1.84. PCR amplification of HLA-DQA1 gene and X-Y homologous gene amelogenin were successful in DNA samples from all 4 groups.

DISCUSSION

Our results demonstrate the following points: (1) urine sediment easily can be used for genetic analysis, (2) urinary sediment can be stored for long periods of time before DNA extraction if it is fixed with ethanol, and (3) DNA obtained from urine can be used for PCR and enzymatic analyses, although DNA from microorganisms such as bacteria, yeasts, or *Trichomonas vaginalis* might be extracted simultaneously with the human cell DNA.

Individual identification and sex determination could be performed using fresh or fixed urine without any difficulty. Reliable extraction of DNA was obtained with 10 mL of urine, even in random single-voiding specimens. Even 5 mL were sufficient if the urine contained epithelial cells or leukocytes. Generally > 100 mL of urine could be collected at one time, and specimen collection was well tolerated by patients. The fixed sediment could be easily transported and stored for at least 2 weeks without evidence of degeneration or fermentation.

DNA extracted from urine was easily used for PCR-based investigations or diagnostic approaches. Although urine may contain proteins that can potentially interfere with PCR, phenol-chloroform extraction can remove substances such as albumin, hemoglobin, and bilirubin.

DNAs from microorganisms including bacteria, yeast, or Protozoa did not result in any problems in PCR amplification. Human sequences were specifically amplified, resulting in HLA-DQA1 and sex determination. Although sperm contamination could cause a misdiagnosis in X-Y identification in women, no actual cases were observed. In the event of such a problem, a microscopic precheck of the urine sediment would prevent this situation.

Our results of HLA typing corresponds well with those of previous reports (5), and the results of sex typing were identical with the registered sex of the donor on the medical record. Our primers for sex determination could amplify both X and Y genes, and the amplified products could be conveniently checked. Urine collection is a much easier procedure than sampling of buccal cells, blood cells, or hair. Additionally, the urine method could be applicable for sex checks in sports events and for individual identification in forensic medicine.

In conclusion, urine can be used as a source material for PCR-based investigation.

REFERENCES

1. Fogazzi GB, Passerini P, Ponticelli C, Ritz E, Cameron JS: Chapter 2 The formed elements of the urinary sediment. Chapter 4 The urinary sediment of the normal subject. In *The Urinary Sediment*, GB Fogazzi, P Passerini, C Ponticelli, E Ritz, JS Cameron, eds. Chapman & Hall Medical, London, 1994, p23–108, 135–137.
2. Maeda M, Murayama N, Ishii N, et al: A simple and rapid method for HLA-DQA1 genotyping by digestion of PCR-amplified DNA with allele specific restriction endonucleases. *Tissue Antigens* 34:290–298, 1989.
3. Nakahori Y, Hamano K, Iwaya M, Nakagome Y: Sex identification by polymerase chain reaction using X-Y homologous primer. *Am J Med Genetics* 39:472–473, 1991.
4. Sada M, Amemiya H, Inoko H: Analysis of enzymatically amplified HLA-DQA1 using restriction fragment length polymorphism. *Transplant Proc* 23:464–467, 1991.
5. Tamaki K, Yamamoto T, Uchihi R, et al: Frequency of HLA-DQA1 alleles in the Japanese population. *Hum Hered* 41:209–214, 1991.