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Urinary Genotyping for DQA1 and PM Loci Using PCR-Based Amplification: Effects of Volume, Storage Temperature, Preservatives, and Aging on DNA Extraction and Typing

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16. Abstract

Urine is often the sample of choice for drug screening in aviation/general forensic toxicology and in workplace drug testing. In some instances, the origin of the submitted samples may be challenged because of the medicolegal and socioeconomic consequences of a positive drug test. Methods for individualization of biosamples have reached a new boundary with the application of the polymerase chain reaction (PCR) in DNA profiling, but a successful characterization of the urine specimens depends on the quantity and quality of DNA present in the samples. Therefore, the present study investigated the influence of storage conditions, sample volumes, concentration modes, extraction procedures, and chemical preservations on the quantity of DNA recovered, as well as the success rate of PCR-based urinary genotyping for DQA1 and PM loci. Urine specimens from male and female volunteers were divided and stored at various temperatures for up to 30 days. The results suggested that sample purification by dialfiltration, using 3,000-100,000 molecular weight cut-off filters, did not enhance DNA recovery and typing rate compared with simple centrifugation procedures. Extraction of urinary DNA by the organic method and by the resin method gave comparable typing results. Larger sample volume yielded higher amount of DNA, but the typing rates were not affected for sample volumes between 1 to 5 ml. The quantifiable amounts of DNA present were found to be greater in female (14-200 ng/ml) than in male (4-60 ng/ml) samples and decreased with the elapsed time under both room temperature (RT) and frozen storage. Typing of the male samples also demonstrated that RT storage samples produced significantly higher success rates than that of frozen samples, while there was only marginal difference in the DNA typing rates among the conditions tested using female samples. Successful assignment of DQA1+PM genotype was achieved for all sampling of fresh urine, independent of gender, starting sample volume, or concentration method. Preservation by 0.25% sodium azide was acceptable for sample storage at 4°C during a period of 30 days. For longer storage duration, freezing at –70°C may be more appropriate. Thus, the applicability of the DQA1+PM typing was clearly demonstrated for individualization of the urine samples.

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Urinary Genotyping for DQA1 and PM Loci Using PCR-Based Amplification: Effects of Sample Volume, Storage Temperature, Preservatives, and Aging on DNA Extraction and Typing

INTRODUCTION

Elimination of drugs from the body involves various processes that occur in the kidneys, liver, lungs, and other organs. Renal clearance is the most important elimination mechanism, which results in the appearance of drugs and their metabolites in the urine (1). Therefore, human urine is frequently analyzed for forensic toxicological evaluations. This is particularly true with workplace drug testing, wherein urine specimens are often submitted because of their easy accessibility (2). In some instances, the integrity of the urine sample may be challenged, and the origin of the sample may need to be verified.

Methods for individualization of the urine samples have largely been based on the indirect interpretation of the expressed genetic markers—that is, individuals are differentiated at the phenotypic level using serological techniques, on the basis of blood cell typing, secretor status, and polymorphic proteins (3). At present, identity testing at the DNA level allows the detection of genetic variation by direct identification of the allelic gene sequences, and the polymerase chain reaction (PCR) has proven to be a powerful tool for this purpose (4). The PCR technique is particularly more effective in analyzing those types of biosamples, wherein the nuclear material is scanty. Although human urinary DNA typing results had been reported (5-8), the effectiveness of the PCR-based DNA technology has not been fully established to characterize the genetic constitution of urine samples: Information about sample requirement, storage condition, preservation, and expected frequency for a successful DNA typing has not been well evaluated. Therefore, the present study was conducted to obtain such information using the PCR-based typing technology.

In the study, different methods of sample concentration and DNA extraction were explored for urine samples collected from human subjects. The effects of storage temperature, storage time, sample volume, and preservatives on the typing efficiency were also investigated. Semi-quantitation of the extracted DNA and typing of the loci for human leukocyte antigen (HLA) and polymarkers (PMs) allowed comparisons to be made among the alternative methods.

MATERIALS AND METHODS

Materials

Reagents, solvents, and other necessary supplies were obtained from commercial sources. All reagents were of analytical grade, and the solvents were of molecular biology grade. The DNA analysis kits (Quantiblot™ Human DNA Quantitation Kit; AmpliType® PM+DQA1 PCR Amplification and Typing Kit) were supplied by Perkin-Elmer Corporation (Foster City, CA).

Sample Collection

All volunteers were fully informed about the study and instructed on the method of mid-stream, clean catch urine collection. Urine specimens were collected in sterile Nalgene® sample bottles and were mixed by gentle inversion for at least 30 min prior to processing, using a hematology mixer. To avoid the effects of repeated sampling and to study the storage effects, samples of each urine specimen were aliquoted fresh into different portions in the appropriate containers prior to the application of different treatment conditions.

Blood specimens were also obtained from the urine donors by finger prick, using sterile lancets and aseptic techniques. Blood samples were processed fresh and served as the subjects' typing reference.

Assessment of Sample Concentration Techniques

An initial assessment on the adequacy of 3 concentration procedures was conducted on urine samples from 8 male volunteers, according to the scheme described in Fig. 1. Male urine samples were selected over female samples as the former samples contain relatively scanty

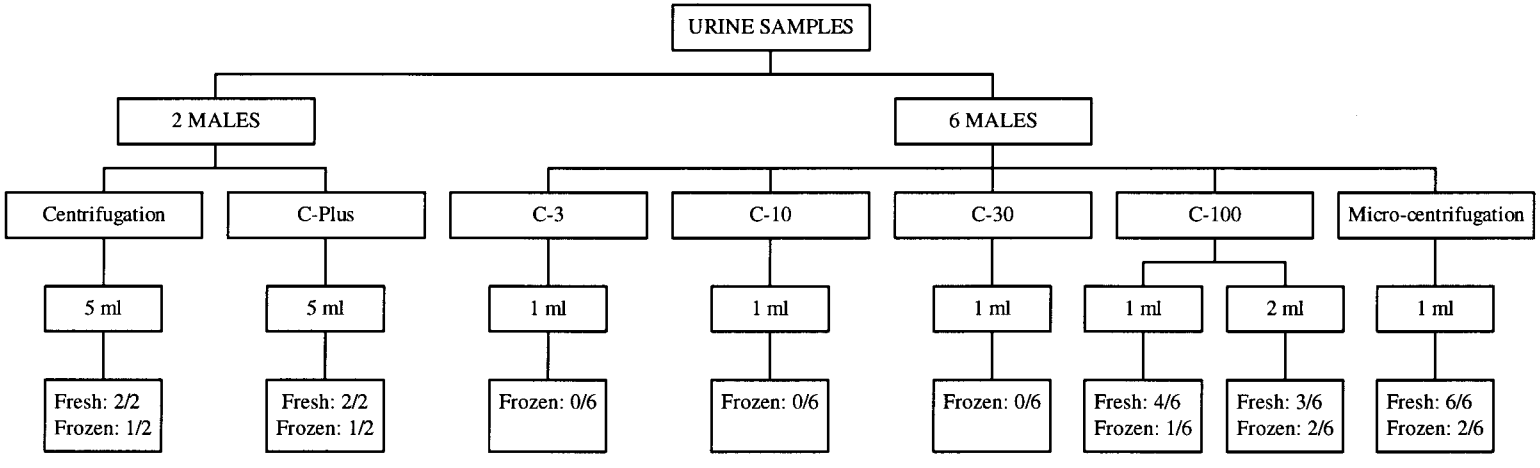

Fig. 1. Preliminary assessment of 3 concentration procedures using fresh and 24-h frozen (-20 °C) urine samples: centrifugation (Procedure 1), micro-centrifugation (Procedure 2), and dialfiltration (Procedure 3) (C-Plus or C-3, C-10, C-30, C-100). These procedures are described in the text. The typeable results reflect the number of samples successfully typed out of the total number of samples tested under a particular category.

amounts of cellular components. Therefore, DNA typing of male urine samples provides a greater challenge for a procedure. The 3 concentration procedures evaluated were centrifugation, micro-centrifugation, and dialfiltration. The obtained urinary concentrates were subsequently used for the DNA extraction.

Centrifugation (Procedure 1)—Each 5-ml sample was concentrated by centrifugation in a centrifuge (GPR Centrifuge, Beckman, Fullerton, CA) at 1,500 x *g* for 15 min in a conical glass centrifuge tube. The supernatant was discarded to the volume of about 0.5 ml, and the pellet was resuspended by gentle vortexing. The suspension was then transferred to a 1.5-ml Eppendorf tube, with 2 volumes of saline rinse (0.9% NaCl), each of 0.3 ml. The obtained suspension was centrifuged at 13,600 x *g* for 5 min (Microcentrifuge 235C, Fisher Scientific Co., Pittsburgh, PA). The supernatant was discarded, leaving approximately 50 µl of the residue.

Micro-centrifugation (Procedure 2)—One-ml urine samples were centrifuged at 13,600 x *g* for 5 min in the micro-centrifuge. After discarding the supernatant layer, a final volume of about 50 µl was retained.

Dialfiltration (Procedure 3)—This filtration was performed according to the manufacturer instruction for Centriplus™ or Centricon™ (Millipore Corp., Bedford, MA). These devices had a molecular weight cut-off (MWCO) covering the range of 3 to 100 kd (Centriplus™-100; Centricon™-3, -10, -30, and -100). Starting sample volumes were dictated by the device capacity, thus 5-ml samples were used with Centriplus™, whereas 1- or 2-ml samples were used with Centricon™. Using a centrifuge with a fixed angle rotor (Ivan Sorvall, Inc., Newtown, CT), ultrafiltering occurred upon the application of the centrifugal force that drives the sample solution through the membrane. The relative centrifugal force was 1,000-5,000 x *g* for 30 to 60 min, depending on the device used. The retained urinary materials larger than the MWCO were recovered by inverted centrifugation. The obtained pellet was resuspended by gentle vortexing. This mixture was transferred to an Eppendorf tube and followed by volume reduction to a final volume of approximately 50 µl, as previously described.

DNA Extraction

Depending upon the nature of a particular experiment, Chelex® 100 resin and/or organic extraction methods were used.

Chelex® extraction—DNA from the urine concentrates was extracted in the presence of Chelex® 100 resin (Bio-Rad Laboratories, Richmond, CA) following the procedure of Walsh et al. (9). To approximately 50 µl of each of the concentrates, 200 µl of the 5 % Chelex® resin suspension in water was added, and the mixtures were incubated in a water bath at 56°C for 30 min and then at 90 to 100°C for 8 min. DNA from whole blood samples was also extracted by this resin method.

Organic extraction—Approximately 50 µl of each urinary concentrate was incubated with 15 µl of proteinase-K (10 mg/ml in deionized water) in 0.5 ml of digest buffer at 56°C for 6 h. DNA was extracted from the mixture by using a chloroform:phenol:isoamyl alcohol system (10).

Comparison of DNA Extraction Methods

Initially, Chelex® 100 resin and organic extractions were separately performed on 1 ml of each of the fresh and 7-day frozen urine samples from 6 male volunteers following concentration by micro-centrifugation (Procedure 2; Fig. 2). The obtained extracts were stored at –20°C until analyzed.

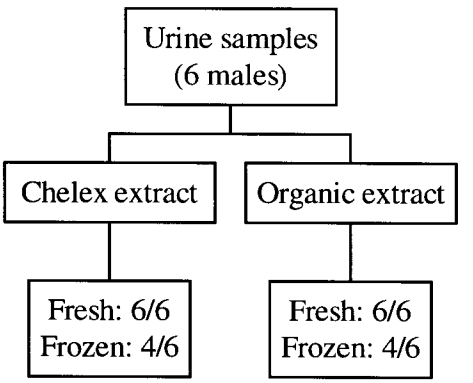

Fig. 2. Comparison of Chelex® and organic extraction methods using fresh and frozen urine samples of male volunteers; the sample volume was 1 ml. Details of the procedures are given in the Materials and Methods section. The typeable results reflect the number of samples successfully typed out of the total number of samples tested under a particular category.

Effects of Sample Volume, Storage Temperature, and Aging

To study the effects of these variables on urinary DNA extraction and typing efficiency, urine specimens from 25 institute volunteers (12 males and 13 females) were used. All specimens were processed immediately, and samples were aliquoted fresh prior to the application of treatment conditions as previously described. Samples were exposed to either room temperature (RT) or -20°C for different time intervals (fresh, 1 day, 2 days, and 7 days). Based on the initial findings, all samples were concentrated by centrifugation (Procedure 1) or micro-centrifugation (Procedure 2) and DNA from the concentrates was extracted by the Chelex® method.

Effects of Preservatives

To the 6 male urine samples, sodium azide (0.25%; w/v) or sodium fluoride (1%; w/v) was added (Fig. 3). At these concentrations, both chemicals act as preservatives. Urine specimens were stored at 4°C and -20°C up to 30 days. After the sample concentration by micro-centrifugation (Procedure 2) and the DNA isolation by Chelex®, DNA samples were amplified and typed.

DNA Quantitation

The amount of DNA in the obtained extracts was determined using Quantiblot™ Human DNA Quantitation Kit. Each 10-µl extract was processed following the recommendations of the manufacturer (11). Chemiluminescent signals were detected on Hyperfilm™-ECL (Amersham Life Science Inc., Arlington Heights, IL) after 30 min exposure and development using a QX-70 medical film processor (Konica Medical Corporation, Wayne, NJ). The intensity of the sample signals was visually compared with that of the DNA standards processed simultaneously. This method selectively determines human DNA semi-quantitatively in the range of 0.15 to 10 ng. Based on the DNA amounts, appropriate volumes of the extracts were utilized in the subsequent DNA amplification.

DNA Amplification and Typing

In vitro DNA replication by *Taq*-polymerase was carried out according to the manufacturer's directions for DQA1 and PM amplification (12). Target regions of 6 genetic loci were amplified from approximately 10 ng of the extracted DNA in a thermal cycler (GeneAmp PCR System 9600, Perkin Elmer Corporation, Foster City, CA) in 32 cycles. Of the 6 loci, 1 locus was human leukocyte antigen (HLA) DQA1. The remaining loci were PM: low density lipoprotein receptor (LDLR), glycophorin A (GYPA), hemoglobin G gammaglobin (HBGG), D7S8 (locus on chromosome 7 linked with cystic fibrosis), and group specific component (GC). All amplified samples were kept at -70°C until typed. Hybridization and detection of the amplified DNA were carried out as per manufacturer's recommendations (12).

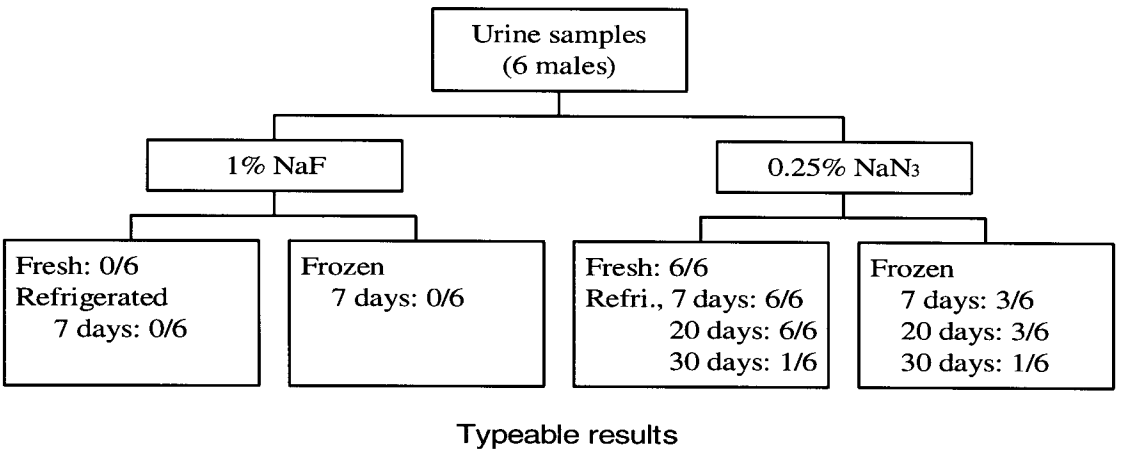

Fig. 3. The typeable results of fresh, refrigerated, and frozen urine samples over a storage period of 30 days in the presence of sodium fluoride (NaF) or sodium azide (NaN_3). Details are given in the text. The typeable results reflect the number of samples successfully typed out of the total number of samples tested under a particular category.

RESULTS

Assessment of Sample Concentration Techniques

Typing results for DQA1 and PM loci suggested that sample purification by dialfiltration (Procedure 3), using Centriplus® or Centricon®, did not improve the typeable frequency relative to other concentration methods (Fig.1). The typeable blots obtained from the dialyzed concentrates showed less intense color reaction than the others, which indicated low amplification efficiency or low concentration of the DNA templates. Therefore, centrifugation (Procedure 1) and micro-centrifugation (Procedure 2) concentration methods were employed in the subsequent experiments.

Comparison of Extraction Methods

As exhibited in Fig.2, the Chelex® resin extracts and organic extracts yielded comparable positive typing rates. However, signals from organic extracts were less intense than that from the resin extracts. Thus, the Chelex® extraction method was selected for further studies. This method is also relatively less cumbersome and less time-consuming.

Amount of Isolated DNA

As is evident from Table 1, the amount of isolated DNA was significantly higher in female than male samples. Also, the DNA amount was slightly higher in the extracts from concentrates obtained by centrifugation (Procedure 1) than by micro-centrifugation

Table 1. The amount of quantifiable DNA in 10 µl of the extracts obtained from concentrates[a] of male and female urine samples at room temperature (RT) and -20°C over a storage period of 7 days.

Sample	Male (n = 12)		Female (n = 13)	
	5 ml	1 ml	5 ml	1 ml
Fresh	83 %[b] (0.20-3.00 ng)	67 % (0.25-3.00 ng)	100 % (2.00->10.00 ng)	100 % (0.70->10.00 ng)
RT (24 h)	67 % (0.20-3.00 ng)	33 % (0.20-2.00 ng)	92 % (0.60->10.00 ng)	100 % (0.60->10.00 ng)
RT (48 h)	42 % (0.15-1.20 ng)	33 % (0.15-0.50 ng)	100 % (0.30->10.00 ng)	100 % (0.50->10.00 ng)
RT (7 days)	33 % (0.20-0.50 ng)	17 % (0.20-0.25 ng)	85 % (0.40->10.00 ng)	92 % (0.15-10.00 ng)
-20° C (24 h)	17 % (0.30-2.00 ng)	8 % (0.15 ng)	77 % (0.15-9.00 ng)	69 % (0.20-9.00 ng)
-20° C (48 h)	8 % (0.25 ng)	8 % (0.15 ng)	62 % (0.15-2.40 ng)	54 % (0.18-4.00 ng)
-20° C (7 days)	25 % (0.20 ng)	8% (0.25 ng)	62 % (0.20-1.25 ng)	69 % (0.15-2.00 ng)

[a] The 2 concentration procedures employed were centrifugation (Procedure 1; 5 ml) and micro-centrifugation (Procedure 2;1 ml). Both procedures are described in the text.
[b]Percentage of samples in which DNA was found to be present within the range given in the parentheses.

(Procedure 2), which could probably be due to the larger initial sample volume used (5 ml *vs.* 1 ml). The DNA content of the samples varied among the subjects. The amount of DNA recovered from 1 ml of fresh urine ranged from 14 to > 200 ng/ml for females and from 4 to 60 ng/ml for males. The quantity of DNA decreased sharply after the first 24 h at frozen storage regardless of the gender: The greater the amount of starting DNA, the greater the decrease. After 24 h, a slower reduction in the quantifiable DNA was observed for up to 7 days. At RT storage, the amount of isolated DNA decreased with the elapsed time. However, the amount of quantifiable DNA from RT samples was significantly higher than that of frozen samples at any given time interval.

DQA1 and PM Typing

The DQA1 and PM typing results (Tables 2 and 3) correlated well with the DNA amounts (Table 1). Results within the male population showed that typeable frequency was sharply reduced after 24 h of storage at –20°C, then remained essentially unchanged at 25% for up to 7 days (Figs. 4 and 5). Samples stored at RT produced significantly higher typing rates than that of frozen samples, independent of concentration methods (Cochran-Q, $p = 0.0001$). For female samples, there was no significant difference in the typing rates among the conditions tested (Cochran-Q, $p = 0.7750$). Although centrifugation (Procedure 1) yielded slightly higher quantifiable DNA than micro-centrifugation (Procedure 2), as explained by the larger sample volume used (5 ml *vs.* 1 ml), such effect was not evident on the typing results. Typeable blots were obtained from samples with DNA content < 0.30 ng/ml, which were below the detectable level by Quantiblot™.

Effects of Preservatives

As depicted in Fig. 3, fresh urine samples with sodium azide were 100% typeable, while all the samples containing sodium fluoride produced negative results. In the presence of sodium azide, typeable results were obtained for all samples stored at 4°C for up to 20 days, while only 3/6 of the frozen samples were typeable. After the 30-day storage in the presence of sodium azide, refrigerated and frozen samples were typed with equal rate of success (1/6).

Table 2. The typeable rates for DQA1 using Chelex® extracts from urine samples subjected to different storage conditions over time.

Sample[a]	Male (n = 12)		Female (n = 13)	
	5 ml	1 ml	5 ml	1 ml
Fresh	100%	100%	100%	100%
RT (24 h)	94%	83%	88%	100%
RT (48 h)	94%	67%	100%	100%
RT (7 days)	71%	50%	100%	92%
-20°C (24 h)	18%	25%	100%	92%
-20°C (48 h)	29%	25%	82%	92%
-20°C (7 days)	35%	25%	76%	92%

[a] Samples were concentrated by centrifugation (Procedure 1; 5 ml) and micro-centrifugation (Procedure 2; 1 ml); RT = room temperature.

Table 3. The typeable rates for PM using Chelex® extracts from urine samples subjected to different storage conditions over time.

Sample[a]	Male (n = 12)		Female (n = 10)	
	5 ml	1 ml	5 ml	1 ml
Fresh	100%	100%	100%	100%
RT (24 h)	100%	92%	100%	100%
RT (48 h)	83%	67%	100%	100%
RT (7 days)	58%	42%	100%	100%
-20°C (24 h)	25%	33%	100%	100%
-20°C (48 h)	25%	33%	100%	100%
-20°C (7 days)	25%	25%	100%	100%

[a] Samples were concentrated by centrifugation (Procedure 1; 5 ml) and micro-centrifugation (Procedure 2; 1 ml); RT = room temperature.

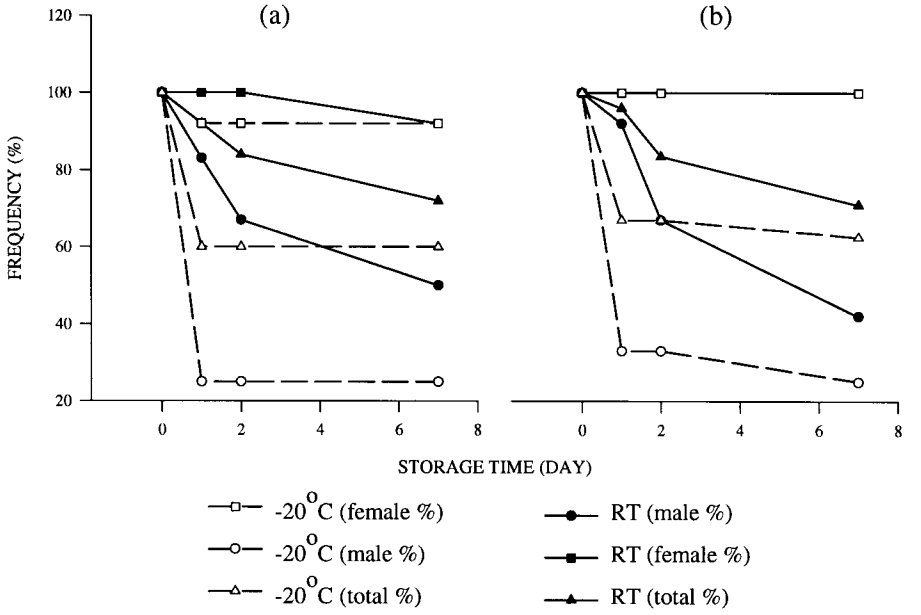

Fig. 4. The typeable frequency for DQA1 (a) and PM (b) markers using female and male urine samples that had been maintained at RT and -20°C for a period of 7 days. (The volume of the urine samples was 1 mL).

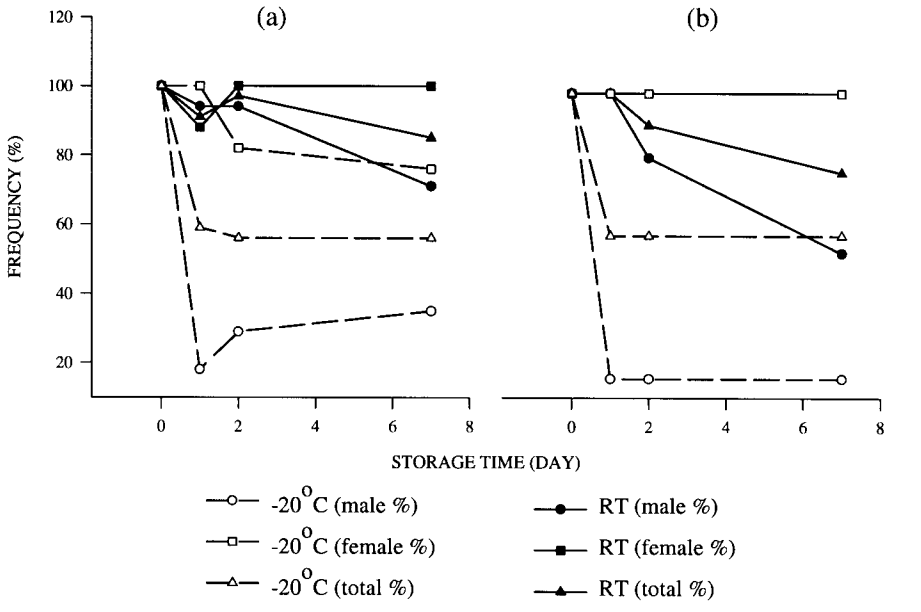

Fig. 5. The typeable frequency for DQA1 (a) and PM (b) markers using female and male urine samples that had been maintained at RT and -20°C for a period of 7 days. (The volume of the urine samples was 5 mL).

DISCUSSION

Because the nuclear material in human urine is scanty and PCR inhibitors are likely to be present (5,7), it was hypothesized that the concentration of samples, as well as elimination of the inhibitors, would improve the DNA amplification efficiency. However, both centrifugation (Procedure 1) and micro-centrifugation (Procedure 2) concentration methods were found to be more suitable for the DNA recovery than the dialfiltration method (Procedure 3). Apparently, the poor DNA recovery by dialfiltration could have resulted from the adsorption of cells and DNA to the filtration apparatus and from the extensive degradation of DNA after freeze-thaw that prevented DNA retention. Alternatively, extractable components from the membrane could have interfered with the amplification efficiency. Of the 3 methods evaluated, micro-centrifugation (Procedure 2) was the method of choice since it produced more consistent results and required a lesser amount of sample. The suitability of the Chelex® resin method over the organic method for the DNA extraction from the concentrates was also reflected in a previous report on the DQA1+PM typing of postmortem forensic tissues and body fluids (13).

Since the epithelial cells from genito-urinary tract and the leukocytes comprise the primary sources of urinary DNA (6), the quantity of DNA varied considerably depending on the physiological state of the subjects—for example, larger amounts of DNA were recovered from female than male urine samples. The present study clearly demonstrated that the DQA1+PM genotype could be successfully assigned for fresh urine, using as little as a 1-ml sample, independent of gender and concentration method. As freezing affected DNA recovery and typing negatively, preservation by freezing is not beneficial for sample storage. It appears that the thawing process damaged DNA, probably through a cell lysis mechanism that would expose naked DNA to environmental insults. Furthermore, the time-dependent effect of freezing on DNA recovery could have resulted from the routine freeze-thaw cycles of the freezer. Thus, storage at -70°C would probably be more appropriate if freezing is required. For frozen urine, the sample may be processed by micro-centrifugation (Procedure 2) in multiple aliquots to increase the DNA yield. Alternatively, the DNA extraction procedure described by Linfert et al. (8) may be employed, as it has been shown to produce 71% typing success. However, the procedure requires 10- to 30-ml urine samples.

The storage duration for urine samples could be further extended by using the chemical preservative sodium azide (0.25%), as storage at 4°C was acceptable for up to 30 days. For longer storage periods, freezing at -70°C is recommended to avoid the deleterious effects of the freeze-thaw cycles of the freezer at higher storage temperature ($\geq$ -20°C). Because sodium fluoride at 1% might have interfered with the PCR, either washing of the urinary residues with saline prior to the extraction or alcohol precipitation of the DNA from the Chelex® extracts did not improve the typeable frequency. Whereas high salt concentration (1% sodium fluoride) in samples may inhibit the PCR, excessive washing could cause additional loss of nucleated cells or further DNA damage. At present, the mechanism of sodium fluoride interference is not clear, but sodium azide may be employed as a preservative for prolonged storage at 4°C. Since sodium azide and sodium fluoride are commonly used as preservatives (5) in urine samples for forensic toxicology/drug testing, the DNA typing can be successfully achieved by knowing the chemical nature of the preservative used in a urine sample.

Overall, this study demonstrated that DNA typing could be effectively applied in resolving the integrity of urine samples submitted for drug analysis. It has applicability not only in postmortem forensic toxicology but also in work place forensic drug testing. However, if the sample is kept frozen for a long duration, then the technique may not be as effective.

REFERENCES

(1) L.Z. Benet, J.R. Mitchell, L.B. Sheiner, Pharmacokinetics: The dynamics of drug absorption, distribution, and elimination, in: A.G. Gillman, T.W. Rall, A.S. Nies, P. Taylor (Eds.), *Goodman and Gilman's The Pharmacological Basis of Therapeutics,* 8th ed., Pergamon Press, New York, NY, 1990, pp. 3-32.

(2) W. Grellner, B. Madea, M. Staak, Individualization of fresh and stored human urine samples by PGM_1- and Gc-subtyping, *Forensic Science International* 68 (1994) 181-93.

(3) L. Kobilinsky, Recovery and stability of DNA in samples of forensic science significance, *Forensic Science Review* 4 (1992) 67-87.

(4) P.M. Schneider, Basic issues in forensic DNA typing, *Forensic Science International* 88 (1997) 17-22.

(5) M.M. Holland, R. Roy, M.D. Fraser, R.H. Liu, Application of serological and DNA methods for the identification of urine specimen donors, *Forensic Science Review* 5 (1993) 1-14.

(6) G.J. Tsongalis, D.E. Anamani, A.H.B. Wu, Identification of urine specimen donors by the PM + DQA1 amplification and typing kit, *Journal of Forensic Sciences* 41 (1996) 1031-4.

(7) I. Medintz, L. Chiriboga, L. McCurdy, L. Kobilinsky, Restriction fragment length polymorphism and polymerase chain reaction-HLA DQa analysis of case work urine specimens, *Journal of Forensic Sciences* 39 (1994) 1372-80.

(8) D.R. Linfert, A.H.B. Wu, G.J. Tsongalis, The effect of pathologic substances and adulterants on the DNA typing of urine, *Journal of Forensic Sciences* 43 (1998) 1041-5.

(9) P.S. Walsh, D.A. Metzger, R. Higuchi, Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material, *Biotechniques* 10 (1991) 506-13.

(10) B. Budowle, J.S. Waye, G.G. Shutler, F.S. Baechtel, Hae III—A suitable restriction endonuclease for restriction fragment length polymorphism analysis of biological evidence samples, *Journal of Forensic Sciences* 35 (1990) 530-6.

(11) Quantiblot™, Human DNA quantitation kit, package insert, Roche Molecular Systems, Inc., Alameda, CA, 1993.

(12) AmpliType®, PM+DQA1 PCR amplification and typing kit, package insert, Roche Molecular Systems, Inc., Alameda, CA., 1995.

(13) A.K. Chaturvedi, N.T. Vu, R.M. Ritter, D.V. Canfield, DNA typing as a strategy for solving issues relevant to forensic toxicology, *Journal of Forensic Sciences* 44 (1999) 187-90.