Forensic Science International: Genetics Supplement Series 8 (2022) 133–135

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/fsigss

Comparisons of protocols for enhanced DNA profile quality from FTA®-deposited urine samples

Korapin Srisiri [a], Tikumphorn Sathirapatya [b], Achirapa Bandhaya [a,*]

[a] Forensic Science Unit, Faculty of Science, Mahidol University, Bangkok, Thailand
[b] Forensic Genetics Research Unit, Ratchadapiseksompotch Fund, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

ARTICLE INFO

Keywords:
Urine
Authentication
FTA® card
Human identification
STR

ABSTRACT

Disputes over the identity of a urine sample donor have been reported, and urine authentication by genetic profiling has helped resolved the cases. However, since genotyping of urine is not always required, many drug-testing laboratories may face sample storage issues. Several studies have investigated the use of FTA® cards as a convenient tool for keeping specimen at room temperature for extended periods of time. However, generating complete STR profile from some FTA®-deposited urine samples remains challenging due to low levels of genetic material content, necessitating amendments to the laboratory's standard protocols. This work therefore aims to evaluate the effects of two DNA template preparation methods, both employing FTA® cards as the storage medium, on the success rates of STR profiling from urine. Specimen from a female volunteer, representing a particularly low-yield sample, was employed. Aliquots of 1 and 2 mL were used as the starting material to evaluate DNA template preparation using the FTA® manufacturer's protocol for disc purification against elution of DNA from the FTA® using Prepfiler™ Forensic DNA Extraction Kit. AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit was used to amplify the STR markers, and the PCR products were analysed using Applied Biosystems™ 3500xL Genetic Analyzer. The DNA profile qualities were examined in terms of number of loci detected and peak height balance. Comparisons with the profiles obtained from DNA isolated using QIAamp® DNA Micro Kit from 1 and 2 mL of the same batch of urine were also made. The optimised protocol was then tested on urine samples from three male volunteers. The results showed that the purification of FTA® punches according to the manufacturer's protocol enabled full DNA profiles to be obtained from both 1 and 2 mL of urine from all samples tested, including male samples. In contrast, no DNA profile could be generated from the DNA eluted with the Prepfiler™ kit. When compared with the more conventional solid-phase DNA extraction method, the profiles generated from the FTA® punches exhibited similar reproducibility and quality to those from the template isolated by the QIAamp® Kit. This work further demonstrated the feasibility of FTA® cards as a tool for specimen storage and DNA template preparation from small volumes of urine for authentication by STR profiling. Full STR profiles could be generated from sample from both sexes without modification of the PCR conditions or injection time.

1. Introduction

Disputes over the identity of a urine sample donor have been reported, and urine authentication by genetic profiling has helped resolved the cases. Although, since genotyping of urine is not always necessary, many drug-testing laboratories may face sample storage issues. Several studies have investigated the use of FTA® cards as a convenient tool for keeping specimen at room temperature for extended periods of time [1,2]. In our previous work, 3 mL was suggested as the minimum volume of urine required to harvest enough genetic material to obtain full DNA profile from both male and female samples [1]. However, processing this amount of urine demands for larger centrifugation equipment or additional steps of aliquoting and combining pellets. Furthermore, studies have reported that extracting the DNA out of the FTA® card matrix could enhanced the quality of STR profiles generated from FTA®-deposited sample [3]. These potential improvements to the previously described protocol were therefore explored in this study to obtain a better DNA profile quality from smaller volumes of urine.

* Corresponding author.
E-mail address: achirapa.ban@mahidol.ac.th (A. Bandhaya).

https://doi.org/10.1016/j.fsigss.2022.10.009
Received 20 September 2022; Accepted 5 October 2022
Available online 6 October 2022

Table 1
Comparisons of the DNA concentration and percentage of alleles detected in STR profiles generated from the challenging female urine sample using different template preparation protocols.

		PrepFiler™ Forensic DNA Extraction Kit	Whatman® FTA® Card	QIAamp® DNA Micro Kit
1 mL	DNA concentration (ng/μL)	Too low	–	0.345 (S.D. = 0.167)
	Alleles detected (%)	0	100	100
2 mL	DNA concentration (ng/μL)	Too low	–	0.628 (S.D. = 0.375)
	Alleles detected (%)	0	100	100

2. Materials and methods

This study was approved by Mahidol University Central Institutional Review Board (MU-CIRB 2022/206.0108).

2.1. Sample collection and preparation

For the protocol comparison experiment, 50 mL of urine from a female whose sample contained very low genetic material (data from [1]) was collected in a sterile tube. For the protocol testing experiment, 50-mL urine samples were collected from three healthy male volunteers. All volunteers were also asked to provide a buccal swab as reference sample. The swabs were extracted using the same protocol as the fresh urine (see 2.3).

Fresh urine was kept in an ice box during transportation to the laboratory and stored at 4°C until use. Each sample was aliquoted into 1 and 2 mL, and two replications from the same batch of urine were performed for each volume and for each protocol in the comparisons. The aliquoted urine was centrifuged at 10,000 × *g* for 10 min [2]. All but approximately 50 μL of the supernatant was discarded and the retained liquid was used to resuspend the pellet.

2.2. Sample deposition on FTA® card and FTA® card purification

The ~50-μL resuspended sample was deposited on Whatman® FTA® card (Sigma) and allowed to air dry overnight. A 2.0-mm puncher size of Harris Uni-core™ (Sigma-Aldrich) was used to punch the sample deposited area of the card. The 2.0-mm disc was placed in a PCR tube. The disc was then purified according to the manufacturer's protocol.

2.3. DNA extraction and quantification

Prepfiler™ Forensic DNA Extraction Kit (Thermo Fisher Scientific) was used to extract DNA from FTA® punches according to the manufacturer's protocol. DNA was extracted from fresh urine using QIAamp® DNA Micro Kit (Qiagen). The extracted DNA were quantified using Qubit™ dsDNA HS Assay Kits and Qubit Fluorometer 3.0 (Thermo Fisher Scientific).

2.4. DNA amplification and data analysis

AmpFℓSTR™ Identifiler™ Plus PCR Amplification Kit (Thermo Fisher Scientific) was used for amplifying STR fragments of the DNA samples. The products were separated and analysed using Applied Biosystems™ 3500xL Genetic Analyzer and GeneMapper™ *ID-X* Software v1.6 (Thermo Fisher Scientific). The number of expected alleles in each profile were determined from the reference sample genotype. The number of detected alleles and heterozygous peak height ratios (PHR) in each profile were calculated.

3. Results and discussion

Data from the DNA preparation protocol comparisons using female urine with low DNA yield as the test material are shown in Table 1.

The results indicated that the Prepfiler™ kit did not perform well in this study, as it failed to elute quantifiable amount of DNA from 2-mm disc of FTA®. This is not surprising, because the amount of DNA loss is expected to increase with the number of steps involved in the DNA preparation. The QIAamp® Micro kit yielded concentrations of DNA that increased with the starting volume of urine, and full STR profiles were produced with balanced PHR (Fig. 1a). Similarly, full DNA profiles with more than 70% PHR were generated from the FTA® punch that had only undergone purification according to the manufacturer's protocol before being added directly into the PCR reagents (Fig. 1b). This finding was different from our previous studies [1], suggesting that the change in the CE platform significantly improved the signal, thus enabled all alleles from the FTA® samples to be detected.

When the entire protocol was tested using 1 mL urine samples from three male volunteers, full DNA profiles with well-balanced peak heights could be generated from all FTA®-deposited samples (Fig. 1b).

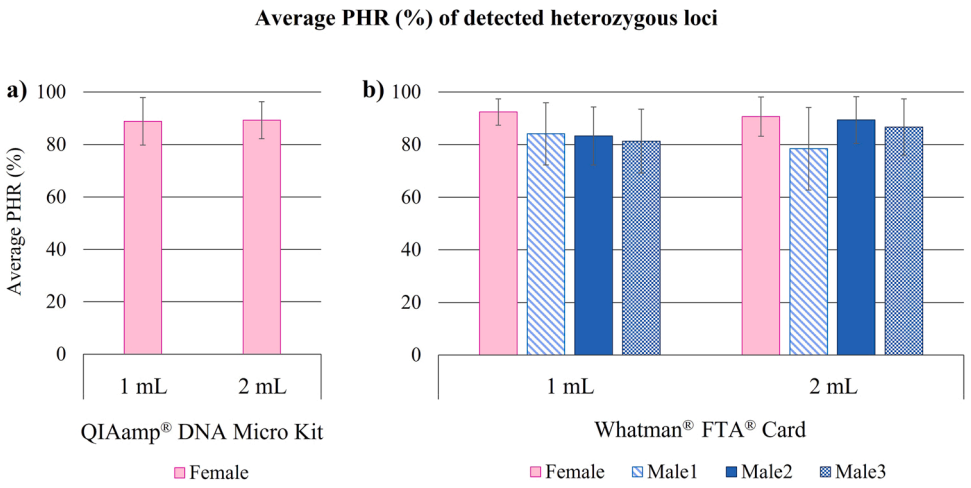

Fig. 1. Average peak height ratios of all heterozygous STR loci detected in a) female urine sample extracted with QIAamp® DNA Micro Kit, and b) male and female urine samples deposited on FTA® that was used as template without eluting DNA from the punch. All profiles were complete, and data presented were calculated from two replicates.

4. Conclusions

This study demonstrated that full STR profile could be generated from a 2.0-mm disc of FTA® card embedded with genetic material from 1 mL of urine without the need for additional extraction step. The profiles obtained had well balanced (>70%) PHR across all heterozygous loci. The entire protocol also seemed to be applicable to urine samples from both sexes and yielded comparable quality of DNA profile to the conventional route of STR genotyping that involves DNA extraction before amplification.

Acknowledgements

The presentation of this work at the 29th Congress of the ISFG was partially supported by the Faculty of Science, Mahidol University. The funding source had no role in any aspect of this study or this paper.

Conflict of interest statement

None.

References

[1] K. Srisiri, R. Jaroenwattana, N. Panvisavas, A. Bandhaya, Optimisation of DNA recovery and analysis of urine samples stored on FTA® card, Forensic Sci. Int. Genet. Suppl. Ser. 6 (2017) e520-–e5522.

[2] P. Grignani, A. Manfredi, M.C. Monti, et al., Genetic individual identification from dried urine spots: a complementary tool to drug monitoring and anti-doping testing, Drug Test. Anal. 14 (2022) 1234–1243.

[3] M. Stangegaard, C. Børsting, L. Ferrero-Miliani, et al., Evaluation of four automated protocols for extraction of DNA from FTA cards, J. Lab. Autom. 18 (2013) 404–410.