



Efficiency of DNA extraction from urine test strips



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ARTICLE INFO

Article history:
Available online 22 October 2015

ABSTRACT

We used six types of urine test strips and cotton swab for comparing the DNA extraction efficiency. DNA extraction was performed with EZ1 Advanced XL, DNA typing with AmpFISTR Identifier Plus PCR amplification Kit, and electrophoresis with the Applied Biosystems 3130xl Genetic Analyzer. Combur-Test, Uriflet S, and UroPaper appear suitable for DNA extraction when using urine test strips as presumptive testing reagents for potential blood.

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1. Introduction

Presumptive testing is a critical process not just in the laboratory, but at crime scenes. Because many articles and materials are left at crime scenes, a key factor in crime-solving is the appropriate assessment of materials early in the investigation process. A presumptive testing reagent must be low cost, highly sensitive, and easy to use. Given these requirements, we assessed the suitability of using urine test strips as presumptive testing reagents for potential blood. At ISALM 2014, we reported on the sensitivity of and the effects of temperature and humidity on five types of urine test strips [1].

In this study, we report on the efficiency of DNA extraction from urine strips.

2. Materials and methods

We investigated six types of urine test strips and a cotton swab (Table 1). The specimen was incubated at 72 °C for 15 min as the digestion step. Extraction was performed using EZ1 DNA Investigator Kit with EZ1 Advanced XL (QIAGEN GmbH, Hilden, Germany), and elution was carried out with 50 µl of TE. DNA quantification was performed with Applied Biosystems 7500 RealTime PCR System (Life Technologies/Thermo Fisher Scientific, South San Francisco, CA, USA) [2], PCR amplification with AmpFISTR Identifier Plus PCR Amplification Kit, and electrophoresis with the Applied Biosystems 3130xl genetic analyzer. Signal intensities exceeding 150 RFU were regarded as valid.

2.1. Study of DNA extraction efficiency

Specimens were prepared so that each urine test strip and cotton swab had 1 µl of each blood sample. After specimen preparation, we extracted DNA using EZ1 immediately or one hour later.

2.2. Study of effects of reagent/blood complex

A pad cut from each urine strip and from a cotton swab was incubated with 200 µl of Buffer G2 + 10 µl of Proteinase K. Following incubation, 1 µl of each blood sample was added to the lysate collected by centrifugation at maximum speed for 1 min using a spin basket (Promega Corp., Madison, WI, USA). The lysate was then incubated. DNA extraction and DNA quantification were performed after incubation.

2.3. Study of effects of reagent in the pad from the urine test strip

The reagent in the pad and DNA from blood, respectively, were extracted using EZ1.

After extraction, each extract from reagent was mixed with DNA extracted from blood, after which DNA quantification was performed.

3. Result

The amount of DNA extracted from urine test strips with blood on the pad was less than the amount of DNA extracted from blood. Except for Hemastix, U-Test Visual, and Uriflet S, the amount of DNA extracted one hour after preparation was nearly equivalent to the amount of DNA extracted immediately after preparation. In the case of Hemastix and U-Test Visual, the amount of DNA extracted immediately after preparation exceeded the amount of DNA

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Table 1
List of urine test strips intended for clinical use.

Urine test strip	Distributor	Detectable range ($\mu\text{g}/\text{ml}^a$)	Detection time (s)
Combur-Test 10UX	Roche Diagnostics Co.,LTD (Minato-ku, Tokyo, Japan)	0.3–7.0	60 [3]
Hemastix	SIEMENS Healthcare Diagnostics (Shinagawa-ku, Tokyo, Japan)	0.15–0.62	60 [4]
Uriase-Kc	Terumo Corporation (Shibuya-ku, Tokyo, Japan)	0.6–7.5	20 [5]
U-TestVisual 5	SANWA KAGAKU KENKYUSHO CO.,LTD (Nagoya, Aichi, Japan)	0.3–10.0	45 [6]
UrifletS-7UA	ARKRAY, Inc (Kyoto-shi, Kyoto, Japan)	0.6–10.0	60 [7]
UroPaperIII 'Eiken'	Eiken Chemical Co., LTD (Shimotsuga-gun, Tochigi, Japan)	0.3–7.5	30 [8]
Cotton swab	JCB Industry LTD (Chuo-ku, Tokyo, Japan)	–	–

^a μg Hemoglobin/ml urine.

Table 2
Results of study of DNA extraction efficiency.

Urine test strip	DNA concentration ($\text{ng}/\mu\text{l}$)	
	One hour later	Immediately
Combur-Test 10UX	0.0243	0.0325
Hemastix	0.0022	0.0053
Uriase-Kc	0.0006	0.0008
U-TestVisual 5	0.0008	0.002
UrifletS-7UA	0.0606	0.0378
UroPaperIII 'Eiken'	0.0266	0.019
Cotton swab	0.0266	0.0319
Blood	–	0.1718

extracted one hour after preparation; in the case of Uriflet S, the amount of DNA extracted immediately after preparation was less than that of DNA extracted one hour after preparation (Table 2).

When we extracted DNA from the reagent/blood complex, the amounts of DNA extracted from Combur-Test, Uriflet S, UroPaper, and cotton swab were nearly equivalent to the amount of DNA extracted from blood alone. The amounts of DNA extracted from Hemastix, Uriase, and U-Test Visual were less than the amount of DNA extracted from blood alone (approximately 1/25, 1/7, and 1/16, respectively) (Table 3).

The effects of the reagent in the pad resulted in amounts of DNA nearly equivalent to the amount of DNA extracted from blood alone.

We obtained the full DNA profile from Combur-Test, Uriflet S, UroPaper, and cotton swab. In the case of Hemastix, DNA typing was successful when we extracted DNA immediately after specimen preparation; when we attempted to extract DNA one hour after specimen preparation, we were unable to obtain the full DNA profile (6/16 loci). In the case of Uriase and U-Test Visual (1/16 and 10/16, respectively), we were unable to obtain a full DNA

Table 3
Results of study of effects of reagent/blood complex.

Urine test strip	Color of mixture	DNA concentration ($\text{ng}/\mu\text{l}$)
Combur-Test 10UX	Light yellow	0.4549
Hemastix	Dark yellow	0.0208
Uriase-Kc	Very light yellow	0.0775
U-TestVisual 5	Light yellow	0.0338
UrifletS-7UA	Yellow	0.403
UroPaperIII 'Eiken'	Very light yellow	0.3067
Cotton swab	–	0.4792
Blood	–	0.5037

profile even when extracting DNA immediately after specimen preparation.

4. Discussion

We compared the DNA extraction efficiency of six types of urine test strips. When DNA extraction is performed with a urine test strip, it would appear to be important to attempt DNA extraction immediately after presumptive testing, since the amount of DNA extracted one hour after preparation would be less than the amount of DNA extracted immediately after preparation.

We performed DNA extraction from a mixture of reagent extract and blood to verify the effects of the reagent/blood complex. The results indicated significant reductions in the amount of DNA extracted with six types of strips when using reagent extract from Hemastix, Uriase, and U-Test Visual. Since reagent in the pad of the urine test strips did not prevent DNA extraction, these results suggest that the reagent/blood complex impairs DNA extraction from urine test strips. Furthermore, there appears to be no relationship between the color of a solution and DNA extraction efficiency, although in certain cases the color of the solution corresponds to the amount of DNA extracted. When the reagent extract from Combur-Test and U-Test Visual is mixed with blood, the color of each mixture changes to the same color (light yellow); this is despite the fact that more DNA is extracted from Combur-Test than from U-Test Visual.

We were unable to obtain a full DNA profile when performing DNA typing using DNA extracted from Uriase and U-Test Visual, probably because the amount of DNA extracted was insufficient.

Our study showed that the amounts of DNA yielded by Combur-Test, Uriflet S, and UroPaper are nearly equivalent to the yield from cotton swabs. In the case of Hemastix, Uriase, and U-Test Visual, the reagent/blood complex prevented DNA extraction, although the reagent in the pad did not prevent DNA extraction. Based on these results, Combur-Test, Uriflet S, and UroPaper appear suitable for DNA extraction when using urine test strips as presumptive testing reagents for potential blood.

5. Conclusion

The reagent in the pad from the urine test strips does not prevent DNA extraction. However, in certain cases, the reagent/blood complex will affect DNA extraction efficiency.

References

- [1] K. Tsukada, et al., Verification study of urine test strips for presumptive testing at crime scenes, *Jpn. J. Legal Med.* 68 (1) (2014) 148.

- [2] K. Tsukada, et al., Influence of presumptive reagents on DNA typing, *Forensic Sci. Int. Genet. Suppl. Ser. 3* (2011) e375–e376.
- [3] Combur Test 10UX package insert, Roche Diagnostics Co., LTD.
- [4] Hemastix package insert, SIEMENS Healthcare Diagnostic.
- [5] Uriace-Kc package insert, Terumo Corporation.
- [6] U-TestVisual 5 package insert, SANWA KAGAKU KENKYUSHO CO., LTD.
- [7] UrifletS-7UA package insert, ARKRAY, Inc.
- [8] Uropaper III 'Eiken' package insert, Eiken Chemical Co., Ltd.