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TECHNICAL NOTE

CRIMINALISTICS

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Specificity, Sensitivity, and Operability of RSID™-Urine for Forensic Identification of Urine: Comparison with ELISA for Tamm-Horsfall Protein

ABSTRACT: In this study, the specificity, sensitivity, and operability of RSID™-Urine, a new immunochromatographic test for urine identification, was evaluated and compared with ELISA detection of Tamm-Horsfall protein (THP). Urine was successfully identified among other body fluids using RSID™-Urine and ELISA detection of THP. The detection limit of RSID™-Urine equated to 0.5 μL of urine; although the sensitivity of RSID™-Urine may be lower than that of ELISA detection of THP, it is thought to be sufficient for application to casework samples. However, results from RSID™-Urine must be interpreted with caution when the sample may have been contaminated with blood or vaginal fluid, because this might inhibit urine detection. The RSID™-Urine assay can be performed in just 15 min by dropping the extracted sample onto the test cassette. Therefore, RSID™-Urine should be an effective tool for the forensic identification of urine, in addition to ELISA detection of THP.

KEYWORDS: forensic science, urine, Tamm-Horsfall protein, immunochromatographic assay, ELISA, body fluid

Urine samples often remain at crime scenes such as sites of murder, harassment, mischief, and sexual assault. At strangulation crime scenes, the location of urine stains may give useful information for identifying where a victim was murdered, because strangulation victims will often become incontinent before dying. In forensic casework, urine stains are characterized by presumptive tests for urea, creatinine, and uric acid (1–6). A new test device for the detection of creatinine, Uritrace™ (Abacus Diagnostics, West Hills, CA), has been made commercially available. However, creatinine is also present in other body fluids such as blood and semen, and urea is not specific enough for urine because sweat also contains relatively high concentrations of urea (7). The determination of uric acid, based on monitoring its UV absorption at 293 nm, is influenced by contaminants such as drugs, which have absorptions near 293 nm. Therefore, it is necessary to develop a more specific and sensitive method for the identification of urine.

Tamm-Horsfall protein (THP) is a high molecular weight glycoprotein, which is a major component of urinary protein (8). Previous reports have outlined the forensic identification of urine stains using radioimmunoassay (9) and enzyme-linked immunosorbent assay (ELISA) for THP (10). In our previous work (11,12), the utility of THP for the forensic identification of urine and urine stains was also evaluated using ELISA and gene expression analysis. It was shown that ELISA detection of THP can be used for the forensic identification of urine; THP was confirmed as a urine-specific protein marker. The simple ELISA method developed in our laboratory can be performed within 5 h using commercially available reagents and is cost-effective.

Recently, a new test for the forensic identification of urine, Rapid Stain Identification of Urine (RSID™-Urine; Independent Forensics, Hillside, IL), has been made commercially available. RSID™-Urine is an immunochromatographic assay that uses polyclonal rabbit antibody specific for THP. The aim of this study was to evaluate the specificity, sensitivity, operability, and cost of RSID™-Urine compared with the ELISA test for the detection of THP which was developed in our laboratory. We also examined the possible interference of body fluids with both tests because urine is often found at crime scenes in mixtures of various types of body fluids.

Materials and Methods

Reagents

RSID™-Urine test cassettes and RSID™-Urine buffer were purchased from Independent Forensics, sheep purified immunoglobulin against human THP was purchased from Biogenesis (Poole, UK) and horseradish peroxidase (HRP)-conjugated rabbit anti-sheep IgG was purchased from Zymed Laboratories (San Francisco, CA). Other reagents used in this study were of research grade and purchased from Wako Pure Chemical Industries (Osaka, Japan).

Samples

Human urine was collected from volunteers as random urine samples. Blood was collected from the brachial vein. Saliva and semen were collected by general noninvasive methods. Vaginal fluid stains were obtained by wiping the vaginal wall with a sterile

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cotton swab. Sweat was collected from the body surface with a polypropylene tube after induction of sweating by physical exercise or sauna use. These samples were stored at −20°C until use. Urine stains, which had been made on a white cotton cloth and stored at room temperature (RT) for 5 years, were used as aged urine stains. Pooled urine samples, prepared from an equal volume of each urine sample obtained from five volunteers, were used to evaluate the sensitivity of RSID™-Urine. All procedures involving human subjects were approved by the Institutional Review Board of the National Research Institute of Police Science.

RSID™-Urine Assay

Each 50-μL portion of undiluted urine or other body fluid was spotted onto a cotton swab and air-dried. In addition, pooled urine was diluted from 1:2 to 1:40 with distilled water and spotted onto a cotton swab. Vaginal fluid stains and aged urine stains were cut into approximately 5 × 5 and 10 × 10 mm squares, respectively. A 50-μL portion of urine was mixed with an equal volume of blood, saliva, semen, or sweat and then spotted onto a cotton swab and air-dried. In addition, 50 μL of urine was spotted onto an approximately 5 × 5 mm sample of vaginal fluid stain and air-dried. The head of a cotton swab or a piece of stain was placed into a 1.5-mL tube and extracted with 500 μL of RSID™-Urine buffer for 1 h at RT with occasional vortexing.

The RSID™-Urine assay was performed following the manufacturer's instructions (http://www.ifi-test.com/pdf/urine_tech.pdf). Each 100-μL portion of extract was added to the sample window. If the extraction efficiency was assumed to be 100%, 100 μL of extract corresponds to 10 μL of body fluid. The result was evaluated on the appearance of visible blue lines at the test [T] and control [C] positions 15 min after the addition of the sample (Fig. 1). Samples with visible blue lines at the test and control positions were considered positive. The intensity of the blue line at the test [T] position was also observed.

Undiluted urine samples and urine samples diluted to 1:10 and 1:100 with RSID™-Urine buffer were also used directly to evaluate the effect of undiluted urine on RSID™-Urine.

ELISA

The vaginal fluid stain was cut into approximately 5 × 5 mm squares and extracted with 100 μL of phosphate-buffered saline. Urine, other body fluids, and vaginal fluid extract were diluted from 1:100 to 1:6400 with 0.05 M bicarbonate buffer (BCB, pH 9.6). Urine was mixed with an equal volume of blood, saliva, semen, vaginal fluid extract, or sweat and diluted from 1:100 to 1:6400 with BCB. Aged urine stains were cut into 10 × 10 mm squares and extracted with 250 μL of BCB for 1 h. These extracts were diluted from 1:2 to 1:128 with BCB.

The ELISA procedure was performed as previously reported (12, 13). Anti-THP and HRP-conjugated anti-sheep IgG were diluted to 1:500 and 1:5000, respectively. Samples with an absorbance value above 0.1 at 490 nm were considered positive.

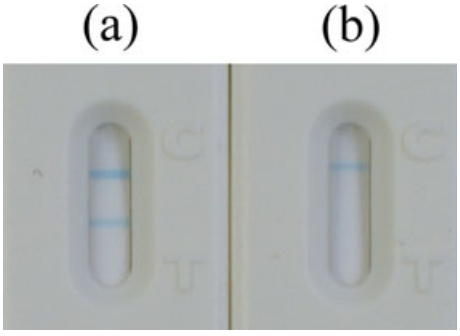

FIG. 1—*Representative results of RSID™-Urine. The sample with visible blue lines at the test [T, lower] and control [C, upper] position was considered positive (a). The sample with visible blue lines at the control [C] position only was considered negative (b).*

Results and Discussion

Specificity

Although the intensities of the test line in RSID™-Urine differed slightly among individuals, all of the six urine samples tested showed positive results (Table 1). The RSID™-Urine urine sample positives were consistent with the results of ELISA detection of THP (Table 1). In addition, the intensities of the test line were correlated to the absorbance values at 490 nm for the ELISA detection of THP. In this study, the volunteers who provided the urine samples were not subject to any special conditions. Although all the samples tested showed positive results, intra- and inter-day variations have been observed in the THP concentration of urine (14). Therefore, factors such as drinking large amounts of fluid may affect the results of RSID™-Urine and ELISA detection of THP.

RSID™-Urine also showed positive results in four of the six aged urine stains (Table 1); however, the intensities of the test line were weak in these samples. In particular, aged urine stain 6 showed a negative result by RSID™-Urine even though it showed a moderate absorbance value in ELISA. The aged urine stains were extracted with 500 μL of RSID™-Urine buffer, although when aged urine stain 6 was extracted with 150 μL of buffer, a positive result was obtained by RSID™-Urine (data not shown). Therefore, to obtain a robust signal, the stain sample should be extracted with a minimal amount of buffer. No positive results were shown for each of the three blood, saliva, semen, vaginal fluid, and sweat samples (Table 2). These results were comparable with those for ELISA detection of THP (Table 2). In contrast, the results of RSID™-Urine were negative for urine samples mixed with blood and vaginal fluid (Table 2). Although the mechanism of this inhibitory effect was not clear, our result was consistent with the

TABLE 1—*Comparison of positive results for urine and aged urine stains between RSID™-Urine and ELISA for Tamm-Horsfall protein (THP).*

	RSID™-Urine	ELISA for THP	
Samples	Result*	Result†	Absorbance at 490 nm
Urine-1	++	+	0.643
Urine-2	+++	+	0.815
Urine-3	+++	+	0.717
Urine-4	++	+	0.539
Urine-5	+++	+	0.838
Urine-6	++	+	0.460
Aged urine stain-1	+	+	0.455
Aged urine stain-2	+	+	0.736
Aged urine stain-3	+	+	0.482
Aged urine stain-4	−	−	0.092
Aged urine stain-5	+	+	0.513
Aged urine stain-6	−	+	0.533

*Appearance of visible blue line at the test or control position 15 min after the addition of sample. The intensity of the test line is recorded as +++, strong; ++, moderate; +, weak.

†Absorbance >0.1 at 490 nm in dilutions of 1:100 for each urine sample or 1:2 for aged urine stains. Positive and negative results indicated by + and −, respectively.

TABLE 2—*Comparison of body fluid specificity between RSID™-Urine and ELISA for Tamm-Horsfall protein.*

	Results	
Body Fluids/Stains	RSID™-Urine*	ELISA for THP†
Blood	−	−
Saliva	−	−
Semen	−	−
Vaginal fluid	−	−
Sweat	−	−
Urine + blood	−	−
Urine + saliva	+	+
Urine + semen	+	−
Urine + vaginal fluid	−	+
Urine + sweat	+	+

*Appearance of visible blue line at the test or control position 15 min after the addition of sample.

†Absorbance >0.1 at 490 nm in a dilution of 1:100 for each sample.

manufacturer's developmental validation study (http://www.ifi-test.com/pdf/UrineValidation.pdf), which indicated that the signal from a urine extract was reduced in the presence of blood. In addition, the result for ELISA detection of THP was negative for urine samples mixed with blood and semen (Table 2). Consequently, RSID™-Urine may be specific for urine and could be applied to aged urine stains in addition to ELISA detection of THP. However, the test result must be interpreted with caution when the sample is potentially contaminated with blood, semen, or vaginal fluid.

THP is highly conserved in mammals; some animal urine samples showed positive results for RSID™-Urine in the manufacturer's developmental validation study (http://www.ifi-test.com/pdf/UrineValidation.pdf). Additionally, canine urine samples showed positive results in the ELISA test for THP (11). Therefore, although RSID™-Urine and ELISA detection of THP may be specific for urine, they cannot be considered as a human-specific test.

Sensitivity

To determine the sensitivity of RSID™-Urine, extracts of serially diluted, pooled urine samples were tested. RSID™-Urine showed positive results, even at a 1:20 dilution of pooled urine. When the extraction efficiency was assumed to be 100%, the detection limit of RSID™-Urine corresponds to 0.5 μL of urine. It was previously reported that the detection limit of THP by ELISA equated to 9.8 nL for a pooled urine sample (12). Although the sensitivities of both tests were not strictly comparable because of differences in the dilution, buffer, and ratio, the sensitivity of RSID™-Urine appears to be lower than that of ELISA detection of THP. However, the sensitivity of RSID™-Urine should still be sufficient for application to casework samples.

The Effect of Undiluted Urine

The manufacturer's instructions indicate that undiluted urine should not be used with RSID™-Urine. To evaluate the effect of undiluted urine on RSID™-Urine, undiluted and diluted urine were tested directly. When undiluted urine was added to the sample window, the transport of the sample fluid was slower than that of diluted urine. In addition, the intensity of the test line was weaker in undiluted urine than in 1:10 diluted urine (Fig. 2). This may be caused by the high-dose hook effect. These results suggested that urine samples should be prepared following the manufacturer's instructions.

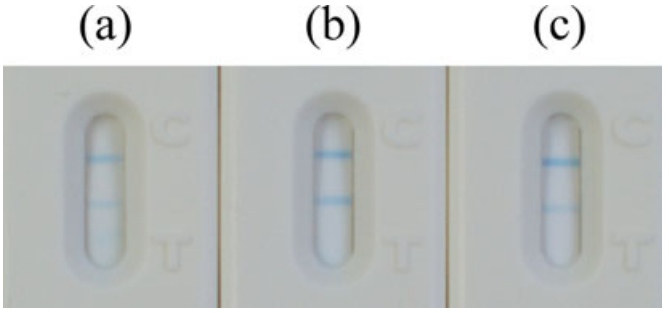

FIG. 2—*The effect of undiluted urine on RSID™-Urine. Undiluted (a), 1:10 diluted (b) and 1:100 diluted (c). Urine samples were added directly to the sample window. The result was observed 15 min after the addition of the sample.*

Operability, Time Requirements, and Cost

The operability, time requirements, and cost were compared for RSID™-Urine and ELISA for the detection of THP. Whereas results can be obtained within 5 h by ELISA, the RSID™-Urine assay can be performed in just 15 min by dropping the extracted sample onto the sample window. Consequently, RSID™-Urine is convenient to use and allows more rapid identification of urine in comparison with ELISA. However, the cost of ELISA is lower (approximately 160¥/sample; $1US = 87.8¥ 2010 average) than that of RSID™-Urine (3600¥/cassette in Japan). Selection of the appropriate test for the forensic identification of urine should take these differences into account.

Conclusions

RSID™-Urine is an effective tool for the forensic identification of urine because it is convenient to use and may be specific for urine among other body fluids. Although the sensitivity of RSID™-Urine appears to be lower than THP detection by ELISA, it should be sufficient for application to casework samples. The effects of various environmental conditions and contamination on the forensic identification of urine by RSID™-Urine or ELISA detection of THP are areas for further investigation.

References

1. Kozu T, Ichinose T, Komatsu Y, Kakegawa K. Identification of urine stains by a urease spray reagent. Rep Natl Res Inst Police Sci 1977;30(1):18–20.
2. Rhodes EF, Thornton JI. DMAC test for urine stains. J Police Sci Admin 1977;4(1):88–9.
3. Ohkuma S. Detection of hydroxybenzaldehydes and ureas with dimethylglyosime and thiosemicarbazide. Yakugaku Zasshi 1955;75(10):1291–2.
4. Gaensslen RE. Sourcebook in forensic serology, immunology and biochemistry. Washington, DC: National Institute of Justice, 1983.
5. Miyamoto N, Omotani N, Wakatsuki R, Kimura S. Identification of body fluid by applying uricase to measure uric acid. Jpn J Legal Med 1976;30:257–8.
6. Wakatsuki R, Kimura S, Miyamoto N, Omotani N. Identification of body fluid by applying uricase to measure uric acid (2nd report). Jpn J Legal Med 1977;31:340.
7. Huang CT, Chen ML, Huang LL, Mao IF. Uric acid and urea in human sweat. Chin J Physiol 2002;45:109–15.
8. Tamm I, Horsfall FL. Characterization and separation of an inhibitor of viral hemagglutination present in urine. Proc Soc Exp Biol Med 1950;74:108–14.
9. Taylor MC, Hunt JS. Forensic identification of human urine by radioimmunoassay for Tamm Horsfall urinary glycoprotein. J Forensic Sci Soc 1983;23:67–72.

10. Tsutsumi H, Okajima H, Sato K, Katsumata Y. Identification of human urinary stains by enzyme-linked immunosorbent assay for human uromucoid. J Forensic Sci 1988;33(1):237–43.
11. Akutsu T, Ikegaya H, Watanabe K, Fukushima H, Motani H, Iwase H, et al. Evaluation of tamm-horsfall protein and uroplakin III for forensic identification of urine. J Forensic Sci 2010;55(3):742–6.
12. Akutsu T, Watanabe K, Fukushima H, Fujinami Y, Sakurada K. Development of a systematic method for identifying saliva, sweat and urine by enzyme-linked immunosorbent assay with statherin, dermcidin and Tamm-Horsfall protein markers. Jpn J Forensic Sci Technol 2011;16(1):1–11.
13. Akutsu T, Watanabe K, Fujinami Y, Sakurada K. Applicability of ELISA detection of statherin for forensic identification of saliva. Int J Legal Med 2010;124:493–8.
14. Kobayashi K, Fukuoka S. Conditions for solubilization of Tamm-Horsfall protein/uromodulin in human urine and establishment of a sensitive and accurate enzyme-linked immunosorbent assay (ELISA) method. Arch Biochem Biophys 2001;388(1):113–20.

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