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Identification of human urine stains on cloth

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Summary. Experimental stains of human urine prepared on 4 different types of cloth and cloth stains in practical cases were examined by 2 methods which have been established in this laboratory. A chemical method determining uric acid and urea nitrogen content and an enzyme-linked immunosorbent assay (ELISA) to determine uromucoid. The former method uses the quotient uric acid/urea nitrogen as an indicator and the latter the quotient ELISA score/protein concentration. All stains were identified as human urine stains by both methods when the specimens were taken from the centre of the stains but when taken from the periphery some of them, especially those on cloth such as cotton or silk, could not be identified as human urine stains. These results indicate that the central part of stains should be preferentially used for the identification of human urine stains, especially those made on cloth such as cotton or silk.

Key words: Urine stains – Uric acid – Urea nitrogen – Immunoassay (ELISA) – Uromucoid

Zusammenfassung. Experimentelle Spuren von menschlichem Urin, auf 4 verschiedenen Typen von Textilgewebe angelegt, sowie Textilspuren aus Routine-Fällen, wurden mit Hilfe zweier Methoden untersucht, welche in diesem Labor erarbeitet worden waren. Es handelt sich um eine chemische Methode, welche die Harnsäure und den Harnstickstoff bestimmt und um einen enzymgekoppelten Immunabsorptionstest (ELISA) zur Bestimmung von Uromucoid. Die zuerst angewandte Methode benutzt den Quotienten aus Harnsäure und Harnstickstoff als Indikator und die letztere den Quotienten aus dem ELISA Score und der Proteinkonzentration. Alle Spuren wurden als menschliche Urinspuren mit beiden Methoden bestimmt, wenn die Proben vom Zentrum der Spuren entnommen wurden. Wenn aber die Spuren von der Peripherie entnommen wurden, konnten einige von ihnen, insbesondere solche auf Stoffen wie Baumwolle und Seide, nicht als menschliche Urinspuren identifiziert werden. Diese Resultate weisen darauf hin, daß der zentrale Teil von Spuren vorzugsweise benutzt werden sollte für die Identifikation menschlicher Urinspuren; besonders gilt dies bei solchen auf Baumwolle und Seide.

Schlüsselwörter: Urinspuren – Harnsäure – Harnstickstoff – Immunoassay (ELISA) – Uromucoid

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Introduction

In forensic science practice, various methods for the identification of human urine stains have been reported [1–11]. Most of them, however, have some drawbacks in their practical application with regards to sensitivity, organ and species specificities, or complexity of the technique. In order to establish a more reliable method, a chemical method using the quotient uric acid (UA)/urea nitrogen (UN) × 20 [12] and an enzyme-linked immunosorbent assay (ELISA) using the uromucoid (S)/protein concentration (P) × 1000 [13] were devised. It was found that the simultaneous use of both methods was useful for the identification of human urine stains. In a previous study [12], it was reported that the UA concentration was very low in the periphery of urine stains on filter paper and should not be used for the chemical method using a quotient UA/UN.

In the present study, the chemical method and ELISA were applied to human urine stains made on various cloths and the effect of the sample location was studied. Furthermore, 3 practical cases were analysed by both methods.

Materials and methods

Materials. Cotton, silk, polyester and nylon were examined because they are commonly used for underwear and bedding. Urine specimens obtained from healthy adults were dropped onto the cloth and the stains were dried for 24 h at room temperature (20° to 25°C). Stains thus prepared were frozen (−30°C) until use. Central and peripheral areas were investigated from each stain. Stains obtained from 3 practical cases were also analysed in this study. Outline of the cases were as follows. Case 1: A 76-year-old woman

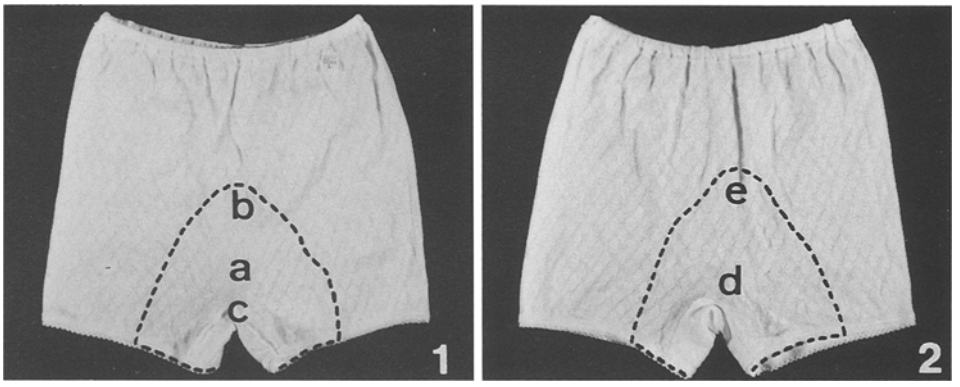

Fig. 1. A stain on the front and the back of the knickers in case 1. *1* front, *2* back

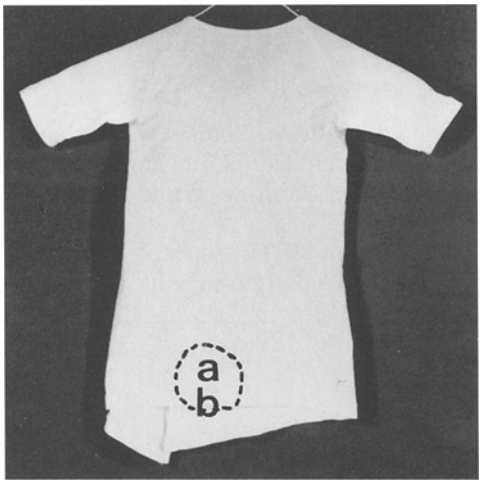

Fig. 2. A stain on the back of the T-shirt in case 2

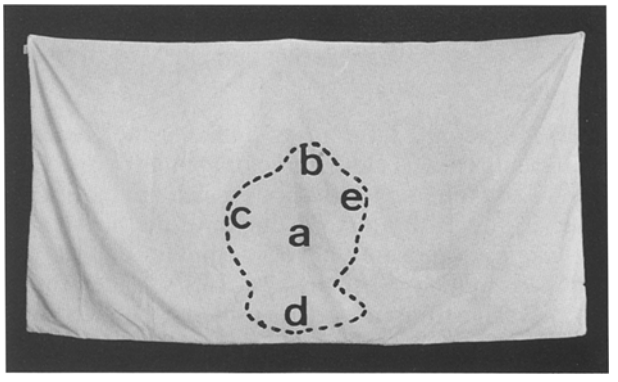

Fig. 3. A stain on the sheet in case 3

was strangled to death. The victim's cotton underwear was examined and a faint brownish stain spread over the front and the back of the knickers was found. The border of the stain is indicated in Fig. 1 by a broken line. Five areas (Fig. 1a–e) were taken from the stain for examination. Case 2: A 3-month-old male baby was strangled to death. The victim's cotton underwear was examined and a faint yellowish stain on the back of the T-shirt was found. The border of the stain is indicated in Fig. 2 with a broken line. Two areas (Fig. 2a and b) were taken from the stain. Case 3: A suspected house-breaker passed urine on the victim's sheet before decamping. On the sheet (polyester 65% and cotton 35%) there was a faint brownish stain indicated in Fig. 3 with a broken line. Five areas (Fig. 3a–e) were taken for examination.

Determination of the UA/UN. UA and UN values were determined by the method described previously [12] using commercial kits; TZ-UA (Tokyo Zouki, Ltd., Tokyo, Japan) for UA and Urea NB-Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for UN. The values of UA/UN × 20 from 50 urine stains from healthy human donors prepared on filter paper were reported previously as 1.11–4.21 [12].

ELISA. Protein concentrations were determined by the method of Lowry et al. [14] and the uromucoid content was determined by a sandwich-type ELISA as described previously [13] using commercial anti-human uromucoid (Cappel Lab., Cockranville, U.S.). The values of the S/P × 1000 from 42 urine stains from healthy human donors prepared on filter paper were previously reported to be > 60 [13]. Most human urine stains gave a score > 20 with a protein concentration lower than 100 μg/ml.

Table 1. Results obtained from experimental urine stains by a chemical method and ELISA

Material	Sample Location	UA (mg/dl)	UN (mg/dl)	$\frac{UA}{UN} \times 20$	S	P (μg/ml)	$\frac{S}{P} \times 1000$
Cotton	Centre	6.15	33.18	3.71	13.6	41.2	329
	Periphery	0	18.61	0	1.1	58.0	19
Silk	Centre	3.59	38.82	1.85	16.6	50.4	328
	Periphery	0.56	26.44	0.43	1.7	37.2	46
Polyester	Centre	1.63	14.14	2.31	11.4	29.0	395
	Periphery	1.84	26.44	1.39	6.8	29.0	236
Nylon	Centre	4.47	64.21	1.39	20.8	46.0	452
	Periphery	4.65	51.53	1.80	21.4	50.6	423

Table 2. Results obtained from urine stains in practical cases by a chemical method and ELISA

Case	Item of clothing	Material	Stain size (cm)	Sample location	UA (mg/dl)	UN (mg/dl)	$\frac{UA}{UN} \times 20$	S	P (μg/ml)	$\frac{S}{P} \times 1000$
1	Knickers	Cotton	35 × 20	Front						
				Centre (a)	2.25	35.24	1.28	5.5	44.8	123
				Periphery (b)	0	48.90	0	2.1	97.4	21
				Crotch (c)	4.52	43.13	2.09	5.6	40.8	137
				Back						
				Centre (d)	3.58	30.31	2.36	3.4	59.8	74
				Periphery (e)	0	37.98	0	2.8	78.6	36
2	T-shirt	Cotton	9 × 8	Centre (a)	15.26	32.70	9.33	26.0	79.0	329
				Periphery (b)	1.59	15.50	2.05	3.0	46.8	64
3	Sheet	Mixed yarn (polyester 65%, cotton 35%)	70 × 50	Centre (a)	5.59	22.17	5.01	12.0	65.8	182
				Periphery (b)	0	0	–	0	16.4	0
				Periphery (c)	0.42	52.06	0.16	2.6	82.4	32
				Periphery (d)	6.05	78.73	1.54	5.0	148.8	34
				Periphery (e)	0.92	49.23	0.37	4.5	127.2	35

Results and discussion

The UA/UN × 20 values and the S/P × 1000 values of the extracts of experimentally prepared human urine stains are shown in Table 1. The UA/UN × 20 quotients from peripheral areas of cotton and silk stains were much lower than those from the central parts and could not be identified as human urine stains. The UA/UN × 20 quotients from peripheral areas of polyester and nylon were similar to those of the central parts and even the specimens from the peripheral areas could be identified as human urine stains. The S/P × 1000 quotients from the peripheral areas of cotton and silk stains showed very low values, stains from the peripheral areas from polyester and nylon gave high values similar to the results on the UA/UN × 20 quotients.

Urine stains obtained from 3 practical cases were analysed by both methods and the results are shown in Table 2. From the UA/UN × 20 values, all stains could be identified as human urine stains when specimens were taken from central parts. However, some stains from the peripheral areas could not be identified as human urine stains. From the S/P × 1000 values, all stains from the central parts could be identified as human urine stains. Specimens from the peripheral areas except the stains in case 2 could not be identified as being of human origin.

These results indicate that the central parts of stains should be preferentially used for the identification of human urine stains, especially those made on cloths such as cotton and silk. It is not exactly understood why different values for the UA/UN × 20 and S/P × 1000 were obtained from peripheral areas of the stains. When urine specimens were dropped onto the cloths the stains on cotton and silk spread more rapidly than those on polyester and nylon and the stains of the former were larger. Therefore, uric acid and uromucoid may easily precipitate when the stains spread diffusely due to low solubility of uric acid in water [15] and high viscosity of uromucoid [16]. The value of UA/UN × 20 obtained from the central part of the stain in case 2 was extremely high (9.33, Fig. 2a) when compared with experimentally prepared human urine stains [12]. The reason why this value was so high has not yet been sufficiently clarified. In addition to the difference in the materials, the cause of death of the victim (strangulation) might be important because the uric acid content of blood increases greatly under anoxic conditions [17–19].

When examining unknown stains, it is important to know their individual characteristics and for the determination of reference substances such as UN for UA or proteins for uromucoid it is very useful to know the complete properties of the stain in question.

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