TECHNICAL NOTE

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Typing of Urine in the ABO and Lewis Systems

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ABSTRACT: A study was conducted to investigate the reliability of determining the ABO type and Lewis group from urine samples stored for prolonged periods under different conditions. Urine samples were successfully typed regardless of storage conditions and storage duration.

KEYWORDS: pathology and biology, genetic typing, urine, ABO blood type determination, Lewis grouping

The U.S. Military currently requires its service members to provide occasional urine samples for drug screening. The results of these tests can be used for criminal prosecution of the alleged sample donor. Since the origin of these samples is under dispute at times, the Army Criminal Investigation Laboratories were asked to develop methods to identify genetic markers in urine to help clarify such situations.

That ABH blood group substances can be found in urine is well established [*1-7*]. They appear to originate from the kidney, bladder, and ureter [*1*]. Lewis substances are also found in urine [*2,8,9*]. No information was found on the viability of A, B, H, and Le^a and Le^b substances in stored liquid urine samples.

Urine contains relatively low amounts of blood group substances as compared to other body fluids [*1,2*]. Concentration of urine samples in Amicon® concentrators (Amicon Corp., Lexington, MA) was recommended by Chase [*10*].

Materials and Method

Since information from the local urine testing laboratory indicated that a maximum of 10 mL of urine would be available for our testing procedures, the AMICON Type A 25 concentrator was chosen for this study: with 0.75 mL it has the lowest fill-volume of the concentrators available to us.

This concentrator not only reduces sample volume up to ×20, but it also removes from the sample substances with a molecular weight of less than 25 000 [*11*] so that marijuana metabolites such as 11-hydroxy-Δ^9-tetrahydrocannabinol, for example, are eliminated from the

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final concentrate [*12–14*]. Blood group substances with a molecular weight range of 3×10^5 to 1×10^6 [*2,15*] are retained.

Urine samples were collected from both male and female laboratory staff over a period of 18 months. Initially, the samples were tested with "Labstix" (Miles Laboratories, Inc.) for the presence of protein, glucose, ketone, and blood. The pH of the samples was determined with a pH meter. The samples were then distributed without any treatment into 7.5-mL test tubes, stoppered, and then stored at room temperature, 4°C, and −20°C.

Since all questioned samples received for analysis contain THC or cocaine metabolites, 10.000-ng/mL Δ^9 THC in methanol was added to 30 urine samples and another 24 samples were spiked with 10.000-ng/mL benzoylecgonine. These samples, from all known donors, ranged in age from fresh to over 18 months of age.

These samples were stored for 24 days at 4°C before being ABO and Lewis typed.

Absorption-inhibition typing for ABH determination was carried out in microtitre plates, while Lewis typing was carried out in small test tubes.

Before the samples were concentrated they were centrifuged for at least 10 min, and without further treatment, 2.25 mL of sample was then gradually placed into the concentrator. This initial sample volume is reduced to 50 to 70 μL. To have sufficient material for two absorption-inhibition tests and (normally) one Lewis typing test, all concentrates were adjusted with physiological normal saline to a final volume of 150 μL. From this "neat" sample, 1:2.5, 1:5, and 1:10 dilutions were prepared.

Absorption-inhibition testing was carried out in microtitre plates by placing 10 μL of the neat concentrate and 10 μL of each of the dilutions into the microtitre plate wells and adding 10 μL of diluted anti-sera (Ortho anti-A: 1:40, Ortho anti-B: 1:70, lab prepared H-Lectin: 1:16). These dilutions were arrived at by titering the anti-sera and the H-Lectin and selecting the next to last dilution giving complete agglutination. The plates were incubated overnight at 4°C. Then 10 μL of 0.5% A(2), B, and O indicator cells were added, incubated for 40 min at room temperature on a rotator, and read microscopically.

For Lewis typing, 20 μL of the "neat" sample were added to 20 μL of diluted anti-sera (Ortho anti-Le^a: 1:3, Ortho anti-Le^b: 1:8) in test tubes. Dilutions were determined as above. Incubation was overnight at 4°C. Then 10 μL of untreated 3% indicator cells were added, incubated at room temperature for 30 min without shaking, then centrifuged for 1 min and read macroscopically.

In each typing test, known A, B, O, and AB secretor urines as well as A, B, and O nonsecretor urine are used as controls. For quality control purposes, a "blind" sample, of which origin and storage conditions are unknown to the examiner, and a urine sample, which is of Lewis type Le^{a-b-}, are included.

Results and Discussion

ABH typing tests were carried out on a total of 219 urine samples ranging in age from fresh to over 18 months of age; on 99 of these Lewis typing was carried out. These samples were collected from 28 individuals. The results obtained are summarized in Tables 1 and 2. It can be seen that both the ABO blood type and the Lewis type could be determined from these samples. There was no instance of obtaining an incorrect result. The only problems encountered were the hemolysis in 1 typing run and the lack of reactivity in some of the samples. It is unknown why no typing reactions were obtained from 3 group "O" samples. These 3 samples were from 1 individual who gave other samples which were typed successfully. It is also unknown why ABH typing reactions were obtained from some of the nonsecretor samples, and not from others. Only 1 individual was available as a donor of group "A" nonsecretor urine and 1 for group "B" nonsecretor urine.

Le^a substance was nearly always found in the urine of secretors in addition to the Le^b substance. The coexistence of these substances in secretors is expected and the same phenomenon is observed when typing saliva or semen.

TABLE 1—*ABH typing tests on a total of 219 urine samples ranging in age from fresh to over 18 months.*

Type	Number Tested	No. of Samples with Reactions for								
		A	A + H	B	B + H	H	ABH	AB	None	Lysis[a]
A, SE[b]	64	38	24							2
B, SE	37			33	4					
O, SE	32					27			3	2
AB, SE	20						7	13		
A, se	20	7							13	
B, se	20			5					14	1
O, se	26								26	

[a]Debris visible in well of microtitre plate.
[b]SE = secretor, se = nonsecretor.

TABLE 2—*Lewis typing on 99 urine samples ranging in age from fresh to 18 months.*

Type	Number Tested	No. of Samples with Reaction for			
		Le^{a-b-}	Le^{a+b+}	Le^{a-b+}	Le^{a+b-}
Le^{a-b+}	54		52	2	
Le^{a+b-}	35				35
Le^{a-b-}	10	10			

Of the samples stored at room temperature and at 4°C, a number soon showed signs of bacterial action, and many had, sometimes heavy, precipitates. Some of the samples stored at room temperature developed a gelatinous consistency and could only be liquified by prolonged centrifugation.

Neither the condition of the sample nor the pH had any obvious influence on the final typing results obtained. The same was true for the few samples in which ketones or proteins were detected. Of particular interest was the fact that even prolonged storage at room temperature did not adversely affect the correct determination of ABO and Lewis types from the urine samples.

The spiked samples were correctly typed. Apparently the presence of these substances does not interfere with the correct ABO and Lewis typing of the urine samples.

Approximately 30% of the samples tested were collected from female donors, at least some of whom are sexually active. Although these urine samples were not tested for the presence of semen, no interference from possible seminal residue was observed. All samples collected were from healthy donors, and the effects of genitourinary infections is unknown.

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