Marilyn G. Chase,[1] *M.Sc.*

ABO Typing Studies on Liquid Urines

REFERENCE: Chase, M. G., **"ABO Typing Studies on Liquid Urines,"** *Journal of Forensic Sciences*, JFSCA, Vol. 31, No. 3, July 1986, pp. 881-885.

ABSTRACT: ABO typing was successfully performed on 46 urine samples whose ABO group and secretor status had been determined previously from blood and saliva. Twenty-four urine samples were collected on which blind studies, time studies, and storage studies were performed. Multiple urines from several individuals were collected to evaluate the duplicity of the test. Also, urines were collected from pregnant and menstruating females to determine if ABO typing was affected under these conditions. Results of these studies are discussed.

KEYWORDS: pathology and biology, genetic typing, urine, body fluids, ABO grouping

Primarily as a result of the recent emphasis on drug urinalysis screening programs, liquid urine samples are being submitted to laboratories for ABO typing in increasing numbers. It is well known that the ABH blood group antigens are found not solely on red cells but in most tissues and secretions of the human body [*1-5*]. However, the amounts found in urine are comparatively low [*2-4*]. This paper describes the method previously reported by Oriol et al. [*5*], used to concentrate urines to a level where the ABH antigens can be detectable by absorption-inhibition procedures. Furthermore, blind trials were performed on urines, and the length of time and conditions under which urines could be stored and still reliably typed in the ABH system were determined.

Collection of Samples

Urine was collected from laboratory donors of known ABO and secretor status. They consisted of a single daytime excretion into a sterile specimen cup which was then stored at 4°C. Several individuals donated more than one urine sample. These individuals supplied samples collected either several hours or days apart. Eight urines were collected from individuals who drank large amounts of fluids. Urines used in the blind trials were collected by a coworker. Each urine was given a code number and stored at 4°C for 48 h. After the blind trials these urines were used for the time and storage study.

Methods and Materials

Typing of urine samples was performed by absorption-inhibition procedures. Some sample preparation was required. From each sample 5 mL of urine were centrifuged at 3400 rpm for 3 min. The supernatant was then concentrated using a Minicon Spinal Fluid Concentra-

Received for publication 3 Oct. 1985; revised manuscript received 19 Nov. 1985; accepted for publication 20 Nov. 1985.

[1]Forensic chemist, U.S. Army Crime Laboratory, Forest Park, GA.

tor CS15 which concentrates 2.5 mL to a 20-fold final concentration. A B15 model may also be used which concentrates 5 mL to a 25-fold final concentration. One drop of the concentrated urine was added to each of three test tubes. Absorption-inhibition was then performed using A and B antisera from Ortho Diagnostics diluted 1:20 and 1:30, respectively. Anti-H lectin was prepared in our laboratory from an extract of *Ulex Europeus* seeds and was diluted 1:13. One drop of antisera was added to each tube and absorption was carried out overnight at 4°C. One drop of a 0.3% red cell suspension was added to each tube. After 10 min at room temperature, the samples were centrifuged and results recorded.

Results

Forty-six urines of known ABO group and secretor status were collected. ABO typing results for these samples are shown in Table 1. Several individuals donated more than one urine sample. The results of ABO typing on these samples are shown in Table 2. Eight of these donors gave a sample after ingesting several glasses of fluids. These results are shown in Table 2.

For the blind trials, a total of 24 liquid urines were collected. These urines were stored for 48 h at 4°C. Absorption-inhibition was then performed. These results are shown in Table 3.

These samples were then divided into two volumes. One half was stored at approximately 26°C (room temperature). Twenty-one samples of the other half were stored at 4°C and three at −20°C (frozen). Absorption-inhibition was performed on these samples at different time intervals. Time studies were conducted until the results were no longer reliable. Figure 1 shows the results of this study. The pH of these samples was also monitored over the storage period. These values are shown in Table 4.

Discussion

This study has confirmed previous reports that ABH antigens can be typed successfully in liquid urines provided the samples are concentrated [5]. Typing results from the same individual can be duplicated in samples collected at different times on the same day, on different days, and after drinking large amounts of liquids. Day-to-day variations in diets of donors did not affect typing results. No ABH antigens were detected in the nonsecretor urines at 20-, 25-, or 50-fold concentrations.

TABLE 1—*ABO typing on urines.*[a]

Samples	ABO Group	Secretor Status	Antigens Detected
13	A	secretor	A, H, or A
8	B	secretor	B, H, or B
16	O	secretor	H
3	B[b]	nonsecretor	none
1	O	nonsecretor	none
2	A[c]	secretor	A, H
1	O[c]	secretor	H
2	A[d]	secretor	A

[a]ABO group and secretor status previously determined on blood and saliva.

[b]The urines from this individual were also concentrated 50-fold.

[c]Pregnant females.

[d]Menstruating females.

TABLE 2—*ABO typing on multiple samples from individuals.*[a]

Donor	Number of Samples	ABO Group	Secretor Status	Antigens Detected	Antigens Detected After Drinking
1	5	A	secretor	A, H, or A	A, H
3	4	A	secretor	A, H, or A	A
4	7	A	secretor	A, H	A, H
5	3	B	secretor	B, H	...
7	2	B	secretor	B, H, or B	B, H
8	2	B	secretor	B, H, or B	B, H
9	3	O	secretor	H	...
10	3	O	secretor	H	...
12	3	O	secretor	H	H
13	3	O	secretor	H	H
14	3	O	secretor	H	H
16	3	B	nonsecretor	none	...

[a]ABO group and secretor status previously determined on blood and saliva.

TABLE 3—*Blind trial ABO typing.*

Number of Samples	ABO Group	Secretor Status	Antigens Detected
11	A	secretor	A, H
3	B	secretor	B, H
10	O	secretor	H

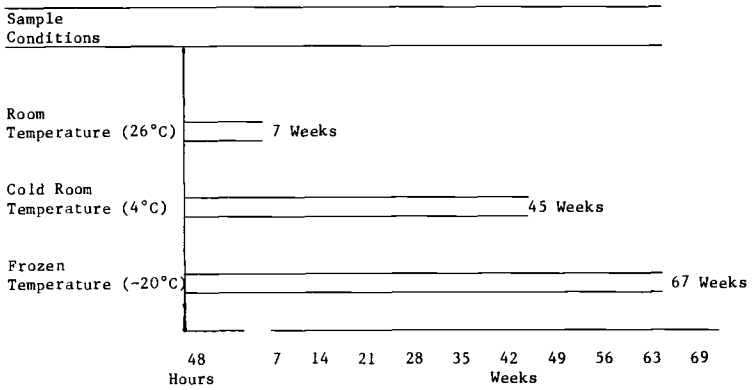

FIG. 1—*ABO typing on stored urines.*

Blind trials were conducted on urines from individuals whose ABO group and secretor status had been established previously from blood and saliva. These were run to evaluate the accuracy and reproducibility of the method.

ABO typing results corresponded to those found in the blood and saliva of all these samples.

TABLE 4—*pH values of stored urine samples.*

	48 Hours	15 Weeks	37 Weeks	67 Weeks		
Sample	4°C	26°C	4°C	4°C	4°C	−20°C
1	6	9	7	7	7	...
2	5	8	6	6	6	...
3	6	8	6	6	6	...
4	6	8	7	7	6	...
5	6	10	6	6	6	...
6	6	7	6	7	...	...
7	6	8	6	7	6	...
8	6	8	7	7	6	...
9	6	8	7	6	6	...
10	6	7	6	6	6	...
11	6	9	6	7	7	...
12	6	8	6	6	6	...
13	7	10	7	6	7	...
14	6	9	7	7	8	...
15	6	7	6	7	8	...
16	7	9	6	7	6	...
17	6	8	5	6	8	...
18	7	9	7	8	8	...
19	6	9	7	8	...	...
20	6	9	...	...	...	7
21	6	8	...	...	...	6
22	6	8	...	...	...	6
23	6	8	6	6	6	...
24	6	7	6	6	6	...

Time studies were conducted to evaluate the effects that time and storage conditions may have on the detectability of ABH antigens found in urine. Urines are routinely typed up to 7 weeks after storage at room temperature and up to 45 weeks after storage at 4°C. The 3 urines kept frozen were typed successfully when this study ended at 67 weeks. Beyond these time periods, for the room and cold temperature urines, weak results or no antigens were detected in numerous samples. No spurious results were observed.

On the limited number of samples tested from pregnant and menstruating females, no variations from expected results were observed.

An increase in pH was seen at 15 weeks in all samples stored at room temperature. As rapid an increase in pH was not observed in urines stored at 4 or −20°C. This increase in pH may be due to bacterial conversion of urea to ammonia which occurs in urines left standing [*6*]. However, there was no correlation between the samples with high pH (9 or 10) and weak or negative ABH typing results.

Age and storage conditions will affect the chances of determining the ABO type of a urine sample. As a result, the history of the sample is necessary before any typing is attempted.

References

[*1*] Race, R. R. and Sanger, R., *Blood Groups in Man*, Blackwell Scientific Publications, Osney Mead, Oxford, 1975.

[*2*] Boorman, K. E., Dodd, B. E., and Lincoln, P. J., *Blood Group Serology*, Churchill Livingstone, New York, 1977.

[*3*] Weiner, A. S., *Blood Groups and Transfusion*, Charles C Thomas, Springfield, IL, 1948.

[*4*] Lee, H. C. and Gaensslen, R. E., "ABO Grouping of Urine and Urine Stains," *Forensic Serology Newsletter*, Vol. VI, No. 3, Sept. 1980, pp. 1-3.

[5] Oriol, R., Cartron, J. P., Cartron, J., and Mulet, C., "Biosynthesis of ABH and Lewis Antigens in Normal and Transplanted Kidneys," *Transplantation*, Vol. 29, No. 3, March 1980, pp. 184-188.
[6] Free, A. H. and Free, H. M., *Urodynamics*, Ames Company, Elkhart, IN, 1974.

Address requests for reprints or additional information to
Marilyn G. Chase
USACIL-CONUS
P.O. Drawer L
Forest Park, GA 30051-1386