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# Concentration of Urine Samples by Three Different Procedures: ABO Typing from Concentrated Urine Samples

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**ABSTRACT:** Urine samples from 28 donors with known blood group and secretor status were concentrated by three different procedures, and ABO typing on the concentrated samples was successfully performed after 12 weeks of storage. The effects of storage with or without sodium azide on ABO typing and on the pH values at several different temperatures were also studied.

**KEYWORDS:** forensic science, genetic typing, urine, ABO typing, concentration, pH

Liquid urine or urine-stained clothing samples are often submitted to forensic science laboratories for ABH blood group antigen determination. Liquid urine samples are also submitted for toxicological purposes. The genetic markers of the urine submitted can be used to determine the origin of any of these samples.

ABH blood group substances have previously been identified from urine [*1–5*]. The source of these antigenic markers appears to be the kidney, bladder, and ureter [*6*].

Since the amount of ABH blood group substance is very low in urine in comparison with other body fluids [*2,6–9*], three alternative procedures were developed for concentration. Concentration by Sephadex® G-200-50[2], Lyphogel® polyacrylamide gels,[3] and Amicon Centricon®-30 microconcentrator[4] was attempted. The concentration procedures were used for detecting ABH antigens from urine by absorption-inhibition and absorption-elution tests.

The effects of addition of sodium azide ($NaN_3$), storage time, and storage conditions on the pH and on the ABH blood group substances were also studied.

## Materials and Methods

Urine samples were collected from 28 donors whose ABO and secretor status were known. A total of 30 mL in samples was collected from each donor from either one or two micturitions in sterile specimen cups. These samples were divided into 5-mL specimens and kept in separate vials. All the specimens were divided into three groups. One

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group of specimens was kept at room temperature (22°C) with $NaN_3$. The second group was stored at 4°C with $NaN_3$, and the third group was frozen at −20°C without $NaN_3$. All the urine samples were stored under the above conditions for a period of 12 weeks. One group of specimens without the addition of $NaN_3$ was also prepared for each group with $NaN_3$.

The pH value of all the samples was determined by a Markson pH meter at the time of collection of the urine and at intervals of 4 weeks. Thus, the pH values of all the samples were monitored at the initial time of collection, at 4 weeks, at 8 weeks, and at the end of 12 weeks of storage.

At the end of 12 weeks, 3 mL of urine from each group was centrifuged at 5000 rpm for 5 min. Two millilitres of the supernatant was then concentrated by one of the following three procedures.

### *Sephadex G 200-50 (Sigma)*

Dialysis bags were prepared from Spectra-por molecular porous membrane (Fisher Scientific No. 08-670-3AA). Membranes were cut into 10-cm sizes. These were boiled in distilled water with 5% sodium bicarbonate for 10 min, rinsed three times with distilled water, and boiled again in distilled water containing 5-mm ethylenediaminetetraacetic acid (EDTA) for 10 min. They were then rinsed twice with distilled water and boiled a third time in distilled water alone for 10 min. After being washed five times with distilled water, the bags were stored in distilled water containing $NaN_3$ and then refrigerated. These bags have a capacity of 0.32 mL/cm and have a molecular weight cutoff of 12 000 to 14 000.

Two millilitres of the supernatant urine sample after centrifugation was put in each dialysis bag. The sample-filled bags were then put in petri dishes (VWR No. 25373-085) individually. The bags were covered with the Sephadex gel beads. Approximately 20.0 g of water is taken up by 1.0 g of the dry gel. Molecules that have molecular weights above 250 000 are excluded from the inner volume of the gel particles. The samples were concentrated until the final volumes of each were approximately 100 μL.

### *Lyphogel (Gelman No. 51030) Polyacrylamide Gel Beads*

Lyphogel gel beads were used as the second method of concentration. These gel beads were dropped directly into the 2-mL supernatant of the centrifuged urine samples. One gram of the Lyphogel beads absorbs 5 g of water from the aqueous urinary samples.

When the samples appeared to be approximately 100 μL in volume, they were centrifuged at 5000 rpm for 5 min. One hundred microlitres of the supernatant aqueous layer was pipetted out for ABH blood group substance determination.

### *Amicon Microconcentrator (Centricon-30)*

Amicon concentrators have been used previously for concentration of urine [*3,4*]. For this study, approximately 2-mL samples from the centrifuged supernatant were used for concentration in Centricon-30 microconcentrators. This concentrator reduces a urine sample volume of 2 mL to approximately 25 μL with a 45° angle rotor and to approximately 40 μL with a 34° angle rotor. It has a molecular weight cutoff of 30 000, so that blood group substances with molecular weights in the range of $3 \times 10^5$ to $1 \times 10^6$ are retained in the final concentrate. For this study, a 35° angle rotor was used and liquid urines were concentrated until the final volume of the concentrate was approximately 100 μL.

### ABH Blood Group Substance Identification from Concentrated Urine Samples

Typing of the concentrated urine samples was performed by both absorption-inhibition and absorption-elution procedures.

For absorption-inhibition, A and B antiserum from Ortho Diagnostics was used. Anti-H lectin was obtained from SERI. The titre of A and B antiserum and of anti-H lectin was determined, and the inhibition procedure was carried out according to a method described previously [*4*]. All the final concentrate volumes of 100 μL from 84 specimens were assayed as "neat" samples.

An alternative procedure for absorption-inhibition was also attempted [*7*] with five of the concentrated samples from each group of concentrate.

For the absorption-elution assay, cotton threads from cheesecloth were used. These were soaked in each of the "neat" samples described for absorption-inhibition assays. The rest of the procedure was followed as described previously [*8,9*].

### Results

ABH blood group substance analysis was performed on all of the concentrated samples by absorption-inhibition and absorption-elution tests. The results are tabulated in Tables 1 and 2, respectively.

The pH values of all the samples were monitored from the time of collection of the urine, at 4 weeks, at 8 weeks, and, finally, at the end of 12 weeks. The pH values of all

TABLE 1—*ABO typing of concentrated urine samples by absorption-inhibition assay.*

| No. of Samples | ABO Group | Secretor Status | Antigens Detected |
|---|---|---|---|
| 10 | A | secretor | A, H |
| 2 | A | secretor | A |
| 3 | B | secretor | B, H |
| 1 | B | secretor | B |
| 7 | O | secretor | H |
| 2 | A | nonsecretor | none |
| 1 | B | nonsecretor | none |
| 2 | O | nonsecretor | none |

TABLE 2—*ABO typing of concentrated urine samples by absorption-elution assay.*

| No. of Samples | ABO Group | Secretor Status | Antigens Detected |
|---|---|---|---|
| 12 | A | secretor | A,(H) |
| 4 | B | secretor | B,(H) |
| 7 | O | secretor | H |
| 2 | A | nonsecretor | none |
| 1 | B | nonsecretor | none |
| 1 | O[a] | nonsecretor | H |
| 1 | O | nonsecretor | none |

[a]Experiments on this sample were repeated, and the same results were obtained.

TABLE 3—*pH values of urine samples after twelve weeks of storage at 22°C, 4°C, and −20°C.*

| Sample No. | Initial Reading | +$NaN_3$, 22°C | −$NaN_3$, 22°C | +$NaN_3$, 4°C | −$NaN_3$, 4°C | −$NaN_3$, −20°C |
|---|---|---|---|---|---|---|
| 1 | 5.5 | 7.3 | 8.5 | 6.0 | 7.0 | 6.0 |
| 2 | 6.7 | 7.0 | 9.0 | 7.2 | 7.9 | 6.7 |
| 3 | 6.0 | 6.8 | 8.3 | 6.0 | 7.0 | 6.0 |
| 4 | 6.0 | 6.0 | 8.7 | 6.2 | 7.0 | 6.1 |
| 5 | 5.5 | 7.6 | 8.3 | 6.5 | 7.8 | 5.4 |
| 6 | 6.8 | 7.8 | 9.0 | 7.2 | 8.0 | 6.7 |
| 7 | 6.9 | 8.0 | 9.5 | 7.0 | 8.0 | 7.0 |
| 8 | 5.4 | 8.0 | 9.9 | 6.0 | 7.2 | 5.5 |
| 9 | 7.0 | 8.0 | 9.8 | 7.0 | 8.3 | 7.1 |
| 10 | 5.6 | 7.6 | 8.7 | 5.7 | 6.5 | 5.6 |
| 11 | 5.6 | 7.6 | 9.0 | 6.0 | 7.0 | 5.7 |
| 12[a] | 7.0 | 9.1 | 10.0 | 7.0 | 8.5 | 7.0 |
| 13 | 6.0 | 7.5 | 8.7 | 7.0 | 7.8 | 6.0 |
| 14 | 6.5 | 7.5 | 8.7 | 7.0 | 8.0 | 6.5 |
| 15 | 6.3 | 7.5 | 8.9 | 7.1 | 8.0 | 6.4 |
| 16 | 6.0 | 7.0 | 8.0 | 6.0 | 7.0 | 6.2 |
| 17[a] | 5.6 | 9.0 | 10.5 | 6.0 | 7.5 | 5.6 |
| 18[a] | 5.5 | 9.0 | 10.2 | 6.0 | 7.2 | 5.5 |
| 19 | 5.5 | 7.7 | 9.0 | 6.0 | 7.0 | 5.8 |
| 20[b] | 7.0 | 7.2 | 9.5 | 8.5 | 9.0 | 7.0 |
| 21 | 6.0 | 6.5 | 7.7 | 7.2 | 8.0 | 6.0 |
| 22 | 7.0 | 7.0 | 8.0 | 7.5 | 8.0 | 7.2 |
| 23 | 7.0 | 7.2 | 8.1 | 7.0 | 8.0 | 7.1 |
| 24 | 6.0 | 6.2 | 7.8 | 7.0 | 7.5 | 6.0 |
| 25[b] | 6.0 | 7.0 | 9.0 | 8.3 | 8.9 | 6.0 |
| 26 | 6.0 | 7.0 | 8.5 | 6.0 | 7.2 | 6.0 |
| 27 | 5.5 | 7.0 | 8.2 | 7.0 | 7.7 | 5.5 |
| 28 | 7.0 | 8.0 | 9.3 | 7.2 | 7.9 | 7.2 |

[a]Experiments on this sample were repeated at different times, and the same results were obtained.

[b]Experiments on this sample were repeated at different times, and the same results were obtained.

three groups of urines stored with or without $NaN_3$ and at different temperatures (22°C, 4°C, and −20°C, respectively) are shown in Table 3.

The inhibition typing results obtained from concentrated samples using all three procedures were successful and corresponded with the blood groups of the donors. The results were the same regardless of the different temperatures and of whether or not $NaN_3$ was used.

Five of the samples from each group of concentrate assayed by the alternate method of absorption-inhibition [7] were also typed successfully (data not shown).

## Discussion

ABH antigens have been successfully typed from liquid urine after concentration by microconcentrators [3,4]. As is evident from this study, ABH blood group substances can be typed successfully from urine samples after concentration with Sephadex G-200-50, Lyphogel, and with the Amicon Centricon-30 microconcentrator. All the concentrated samples, using the three alternative procedures, retained blood group substances in their final concentrate volumes of 100 μL.

No erroneous results were obtained from any of the 28 samples processed. No blood

group substances were detected in nonsecretor urines by the absorption-inhibition assay even after 40-fold concentration. Only one sample (O, nonsecretor) indicated the H blood group substance by the more sensitive absorption-elution assay.

Only five nonsecretor individuals were available for this study. A larger nonsecretor pool would have been preferable but was unavailable. AB individuals (secretors and nonsecretors) were also unavailable.

As is evident from the data in Table 3, samples stored without $NaN_3$ at room temperature and at 4°C showed an elevated pH compared with their counterparts stored with $NaN_3$. Changes in the pH value did not affect the concentration or the results of ABH blood group substance analysis

When kept at room temperature, Samples No. 12, 17, and 18 showed a higher pH value and more precipitate at the end of twelve weeks than did the other samples. $NaN_3$ added to the samples stored at room temperature and at 4°C possibly reduced the bacterial growth. Bacterial conversion of urea to ammonia has been reported to occur in urine samples stored at room temperature [*10*]. If $NaN_3$ were ineffective in preventing bacterial growth, this might have contributed to the increase in these samples' pH values.

The procedures described above for concentration of urine have been used in this laboratory for successfully concentrating other body fluids such as blood, semen, and saliva. Genetic marker analyses have been successfully performed from these concentrated body fluid samples. The results of this study will be published at a later date.

Forensic science laboratories routinely receive urine samples for toxicological and serological analysis. Since blood group substances are difficult to obtain from urine, procedures for successful concentration and blood group substance analysis would be extremely useful. This study also indicates that aged urine samples brought in as evidence can be typed successfully after concentration.

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