A Simplified Alkaline Phosphotungstate Assay for Uric Acid in Serum

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An alkaline phosphotungstate procedure was developed in which the phosphotungstic acid reagent is used both as protein-precipitating and oxidizing reagent. The procedure requires only a 0.2-ml sample and fewer reagents and manipulations than similar procedures, but yields results equivalent to those obtained by Henry's alkaline phosphotungstate procedure. Recovery, precision, and linearity were good over a wide range of uric acid concentrations. Standardization was easy with use of aqueous albumin standards or of commercial calibration reference sera.

Additional Keyphrases nonspecific chromogens • ascorbic acid • commercial reference sera

Uric acid is most commonly determined by the alkaline phosphotungstate method of Folin-Dennis or one of its numerous modifications (1–3). Application of most of these methods is limited by turbidity, nonspecificity, or poor uric acid recovery.

Incomplete recovery of uric acid is a problem common to most uric acid methods in which protein-free filtrates are used. Uric acid usually coprecipitates with the proteins, especially as the pH is decreased (4). However, uric acid can be determined without protein precipitation by use of the specific enzyme, uricase, the decrease in absorption being measured at 295 nm (5). Henry (4, 6) compared two phosphotungstate methods and a uricase spectrophotometric method, and found that results by the three methods correlated highly.

We describe here a uric acid procedure in which a single addition of phosphotungstic acid is used both as protein-precipitating and oxidizing reagent. The procedure requires 0.2 ml of serum, and fewer reagents and manipulations than similar methods.

Materials and Methods
Reagents
(a) Phosphotungstic acid reagent. Dissolve 40 g of molybdenum-free sodium tungstate in about 300 ml of distilled water. Add 32 ml of orthophosphoric acid (850 g/liter) and reflux gently for 4 h. Cool to room temperature and add about 300 ml of distilled water. Add 32 g of lithium sulfate monohydrate and dilute to 1 liter with distilled water. Add 81 ml of 2N sodium hydroxide to 1 liter of the phosphotungstic acid solution. The final pH should be 2.5 ± 0.1, and the solution should be clear and slightly greenish yellow.

(b) Sodium carbonate solution. Sodium carbonate (anhydrous), 140 g/liter.

Procedure
Add 0.2 ml of sample to 2 ml of reagent a and mix thoroughly. Let stand 15 min at room temperature. Centrifuge; immediately and completely decant the supernatant solution into a clean tube. Add 1 ml of reagent b and mix thoroughly. Let stand 15 min at room temperature. Measure the absorbance of the sample vs. a reagent blank at 700 nm.

The reagent blank should be practically colorless, and the final color is stable for at least 1 h.

Standardization. Aqueous uric acid standards should be prepared in albumin solution (about 50 g/liter). The assay procedure may also be standardized with calibration references sera such as “Versatol” and “Calibrate” (both from General Diagnostics Division).

Results
Phosphotungstic Acid Reagent Modification
Phosphotungstic acid was prepared originally as described by Henry (4, pp 278–280). We added various quantities and normalities of sodium hy-
droxide to this reagent to obtain a protein-free filtrate having a pH at or near 3. However, insufficient final color was obtained with samples having high uric acid concentrations (>9 mg/100 ml) and relatively high protein concentrations (8 mg/100 ml). Presumably, after phospho-18-tungstic acid had precipitated the protein insufficient reagent was left to completely oxidize the uric acid. This was corrected by a 2-h increase in the reflux time used to prepare Henry’s phosphotungstic acid reagent. The pH of the protein-free filtrate obtained with the modified reagent ranged from 2.60 to 2.73. Uric acid recovery was apparently unaffected by this relatively low pH because the uric acid is oxidized before precipitated proteins are separated.

Evaluation

Specificity. Commercial calibration reference sera, day-old hospital sera, and freshly drawn normal sera were assayed, treated with uricase, and then reassayed. Uricase treatment consisted of incubating 3 mg of uricase (activity, 2.8 U/ml) per ml of serum for 20 min at 25°C. All of the uric acid was destroyed in the commercial and hospital sera; there was no extraneous color after treatment. Fresh normal sera, however, exhibited a final absorbance equivalent to 0.6 to 1.2 mg of uric acid per 100 ml, and an occasional sample gave a final absorbance equivalent to as high as 1.6 mg of uric acid per 100 ml. Similar results were obtained by Henry’s method (4, pp 278–280). Thus, although the uric acid was presumably destroyed when fresh normal sera were treated with uricase, the absorbances associated with fresh normal sera apparently reflected the presence of relatively unstable nonspecific chromogens. Ascorbic acid is probably the most important such chromogen, since it would be present in fresh sera but not in day-old or commercial serum calibration references, and we obtained evidence that this is so: Physiological amounts of ascorbic acid were added to Versatol before uricase treatment, which was then assayed by the proposed and uric acid procedure and that of Henry. In both assays, the final absorbance of the Versatol samples was equivalent to that found with the normal sera (0.4 to 1.2 mg of uric acid per 100 ml).

Recovery studies. Lyophilized Versatol and Versatol-A Alternate were reconstituted with appropriate uric acid solutions so that there was an additional 1, 2, or 3 mg of uric acid per ml more than the labeled values. Recovery of this added uric acid was 93 to 103% and 98 to 103% with the Versatol and Versatol-A Alternate samples, respectively (Table 1). A recovery study of aqueous acid standards in solutions of albumin (50 g/liter) is also summarized in Table 1.

Table 1. Recovery of Uric Acid Added to Commercial Reference Sera or to Albumin Solution (50 g/liter) Containing 50 mg of Uric Acid per Liter

<table>
<thead>
<tr>
<th>Sample</th>
<th>Uric acid added mg/100 ml</th>
<th>Expected value mg/100 ml</th>
<th>Value found mg/100 ml</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Versatol</td>
<td>1</td>
<td>4.00</td>
<td>4.03</td>
<td>1.03</td>
</tr>
<tr>
<td>Versatol</td>
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<td>5.00</td>
<td>4.96</td>
<td>1.96</td>
</tr>
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<td>Versatol-AA</td>
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<tr>
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<td>8.30</td>
<td>8.27</td>
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<tr>
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<td>9.05</td>
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</tbody>
</table>

Fig. 1. Uric acid standard curve

- Uric acid standards in aqueous albumin, 5 g/liter. Reference sera: ○ “Calibrate-1”, 3 mg/100 ml; • “Calibrate-2,” 6 mg/100 ml; □ “Calibrate-3,” 9 mg/100 ml; △ Versatol, 3.1 mg/100 ml; Δ Versatol-A Alternate, 5.1 mg/100 ml; Π Versatol-A, 8.3 mg/100 ml

Standardization. The standard curve with Versatols and Calibrates was linear to 20 mg of uric acid per 100 ml. Aqueous uric acid standards cannot be used directly because of the volume lost during decantation, by entrapment in the protein precipitate. However, aqueous uric acid standards may be used if they are dissolved in albumin solution as described. The standard curve, with commercial serum calibration references and with aqueous standards containing albumin, is shown in Figure 1.

Comparison studies. The proposed uric acid procedure was compared with Henry’s alkaline phosphotungstate procedure. Thirty-eight fresh normal sera (from males and females, nonfasted) gave a mean of 5.4 ± 1.0 (±s) and a range of 3.4 to 7.0 mg/100 ml by the proposed procedure, and 5.3 ± 1.1 (±s), range 3.0 to 7.0 mg/100 ml, by
Ascorbic acid and other reducing constituents in samples of freshly drawn blood affect the results by our proposed procedure essentially the same as they affect those obtained by Henry's alkaline phosphotungstate procedure. Jung and Pareth (8) claimed to have eliminated all nonspecific chromogens by treatment with trisodium phosphate before the protein is precipitated. We found that the treatment with trisodium phosphate in their or our procedure did not eliminate the interfering nonspecific chromogens in Versatol containing 1, 2, or 3 mg of ascorbic acid per 100 ml. The contribution of the nonspecific chromogens may be accounted for in our procedure by assaying a sample with and without a uricase treatment. Because most uric acid determinations are not done under emergency conditions, the major nonspecific chromogens can be eliminated conveniently by allowing the sera to stand refrigerated overnight.

Good recovery, precision, and linearity were achieved with the proposed uric acid procedure over a wide range of uric acid concentration, and either aqueous uric acid standards containing albumin or calibration reference sera could be used in standardizing. The phosphotungstic acid reagent used in the proposed assay was effective as both protein-precipitating and oxidizing reagent. Fewer reagents and manipulations, and less sample, are required and results are equivalent to those obtained with Henry's alkaline phosphotungstate procedure for uric acid.

References