Evaluation of Determination of Uric Acid in Serum and Whole Blood with the Reflotron®

G. Cattozzo,1,2 C. Franzini,1,2 A. Hubbuch,3 and W. Tritschler3

The performance of the Reflotron system (Boehringer Mannheim) for the determination of urate in whole blood and serum was evaluated. Within-run and day-to-day imprecision of the system were comparable with those for a solution-chemistry enzymatic method (overall CVs in the range 2.2–2.5%). Results for 100 individual specimens with urate concentrations ranging from 16 to 134 mg/L agreed well with the comparison method, both for serum and whole blood. We saw no significant interference from lipemia or hemoglobin. Bilirubin interfered at concentrations >100 mg/L. Hematocrit variation between 25% and 55% did not affect results for whole blood; variation of the applied sample volume from 28 μL to 35 μL (stated sample volume requirement: 30 μL) did not significantly influence the measured value. We consider results produced by the system to be of the same analytical quality as those obtained by the more conventional solution-chemistry methods that are currently available.

Additional Keyphrases: enzymatic methods • reflectance photometry • physician’s office testing

The Reflotron system (Boehringer Mannheim, Mannheim, F.R.G.), based on the dry-chemistry approach, is used for substrate and enzyme assays of analyses in whole blood, plasma, or serum. This is made possible by the unique plasma separation device integrated into the reagent carrier. The system has been described elsewhere (1–3) and its performance in the assay of alanine aminotransferase and gamma-glutamyltransferase in whole blood has been recently assessed (4). We report here an evaluation of the system’s performance in determining urate in serum and whole blood.

Materials and Methods

The basis for urate determination as implemented in the system (Reflotron Uric Acid, cat. no. 745 103) is the uricase/catalase (EC 1.1.1.17)/chromogen acceptor reaction (5). The oxidized form of the chromogen, 4-[4-[4-(dimethylamino)phenyl]-5-methyl-1H-imidazol-2-yl]-2,6-dimethoxy phenol hydrochloride, has very high molar absorptivity, 3700 m² mol⁻¹, at 642 nm. The system is factory-calibrated on the basis of nine pooled sera to which urate concentration values (range: 22–178 mg/L) were assigned by means of the comparison method (see below) according to an established protocol (6, 7). Each Reflotron is used to assay these sera in 10 replicate assays each and the calibration curve, as a plot of the percentage of reflectance (R) vs urate concentration, is fitted to the Kubella–Munk equation [c = A(0) + A(1)R + A(2)R⁻¹, with A(0) = 6.12161; A(1) = -7.13674; and A(2) = 3906.33]. The %R values generally span the range 20 to 65. The equation for the curve, encoded in the magnetic area on the reverse side of the reagent carrier, is read automatically during each assay with the system.

In the comparison method, we used a manual uricase/catalase (EC 1.11.1.6)/Hantzsch’s condensation method (Urica-quant®; Boehringer Mannheim) with individual sample blanks (8), a high-precision diluter (Dilutrend; Boehringer Mannheim), and a Beckman Model 42 spectrophotometer.

Evaluation of within-run and day-to-day imprecision: Two lyophilized control sera ("Precinorm U" and "Precipath U"; Boehringer Mannheim) and two pools of fresh patients’ sera (low- and high-concentration pools), aliquoted and stored frozen for a week, were assayed on five consecutive days, with 10 replicate assays each day, by both the Reflotron and the comparison method.

Measuring range and linearity: Nine intermediate graded mixtures were prepared from a low-concentration (22 mg/L) and a high-concentration (139 mg/L) serum pool. Each mixture and the two original pools were assayed with the reagent carrier and the comparison method in five replicate measurements each.

Method comparisons: We collected 100 venous blood specimens in two aliquotes: one with heparin anticoagulant and one with none. Serum specimens were assayed with the Reflotron Uric Acid method and with the comparison method, whole-blood specimens only with the Reflotron method. We also assayed, with the Reflotron, capillary and venous blood obtained from 50 additional patients.

Interference studies: Effects of hematocrit and lipemia were investigated as part of the comparison studies. Interference from hemoglobin was studied by adding increasing amounts of hemolyzed to plasma up to a maximum concentration of hemoglobin of 5 g/L, and assaying. Four blood specimens from four donors with uric acid concentrations ranging from 39 to 58 mg/L were assayed in this experiment. We tested interference from bilirubin by mixing two fresh serum pools (4 mg/L bilirubin/73 mg/L uric acid and 320 mg/L bilirubin/65 mg/L uric acid) to prepare 12 specimens, and assaying. We measured each sample in the interference studies in sextuplicate.

Sample volume: We studied the effect of varying sample volume between 25 and 35 μL with six replicates of plasma and blood specimens.

Statistical analysis included: ANOVA (9) for evaluating imprecision, to estimate the "pure" day-to-day variation; linear regression analysis (least squares, standard model) for evaluating linearity; and nonparametric linear regression (10) for evaluating the method comparisons.

Results

Statistical evaluation of analytical imprecision is shown in Table 1 for total imprecision and for within-run and day-to-day imprecision. Statistical assessment of linearity (range 22–139 mg/L) as regression/correlation analysis of mean found values (y) vs the volume fraction of the high-concentration pool (x) is shown in Table 2 for the two analytical methods.
Table 1. Imprecision of Reflotron Uric Acid and the Comparison Method

<table>
<thead>
<tr>
<th>Component of variance</th>
<th>n</th>
<th>PN</th>
<th>PP</th>
<th>LP</th>
<th>HP</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>50</td>
<td>2.2</td>
<td>2.2</td>
<td>2.4</td>
<td>1.9</td>
<td>2.5</td>
</tr>
<tr>
<td>Day-to-day</td>
<td>5</td>
<td>0.2</td>
<td>0.5</td>
<td>0.7</td>
<td>0.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Overall</td>
<td>50</td>
<td>2.2</td>
<td>2.3</td>
<td>2.5</td>
<td>2.0</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Mean concn, mg/L

<table>
<thead>
<tr>
<th></th>
<th>PN</th>
<th>PP</th>
<th>LP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-run</td>
<td>64.6</td>
<td>80.6</td>
<td>29.5</td>
<td>79.3</td>
</tr>
<tr>
<td>Day-to-day</td>
<td>78.7</td>
<td>28.8</td>
<td>80.3</td>
<td></td>
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</tbody>
</table>

ANOVA was used to calculate overall imprecision, resolved into the within-run and day-to-day components. PN and PP; Precinorm U and Precipath U; LP and HP; serum pools with low and high concentrations of urate.

Table 2. Assessment of Linearity: Regression/Correlation Analysis of Urate Concentrations (y) vs Volume Fraction (x)

<table>
<thead>
<tr>
<th>Urate concn range, mg/L</th>
<th>y-intercept, mg/L</th>
<th>Slope</th>
<th>S_yx mg/L</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflotron</td>
<td>27.9–139</td>
<td>26.9</td>
<td>11.1</td>
<td>0.9995</td>
</tr>
<tr>
<td>Comparison method</td>
<td>27.4–138</td>
<td>26.7</td>
<td>11.0</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

n = 11 each

Results of the method-comparison experiments are as follows (concentration values in mg/L):

- For serum: Reflotron (y) vs comparison method (x): y = -1.66 + 1.03x; r = 0.9990; S_yx = 1.79; range = 15.0–135; n = 100.
- Reflotron (whole blood) (y) vs comparison method (serum) (x): y = 1.62 + 0.94x; r = 0.993; S_yx = 1.68; range = 15.0–135; n = 100.
- Reflotron (capillary whole blood) (y) vs Reflotron (venous whole blood) (x): y = 0.88 + 0.99x; r = 0.991; S_yx = 1.77; range = 30.8–136; n = 50.

We saw no interference from hemoglobin for hemoglobin concentrations up to 2.5 g/L. At 5 g/L, there was mild negative interference (values 5% lower than expected) in some but not all the specimens tested. Bilirubin gave positive interference, linearly correlated with its concentration: measured concentrations of urate were about 5% and 10% greater than the true value at bilirubin concentrations of 70 and 140 mg/L, respectively.

Results for hyperlipemic sera (cholesterol concentration up to 4.8 g/L and triglyceride concentration up to 12.4 g/L) by both methods were the same as for normolipemic sera: no correlation was evident between intermethod differences for individual specimens and cholesterol or triglyceride concentration. Influence of the hematocrit value on results obtained with whole blood was not evident in the method-comparison experiments, in which we assayed 100 specimens with hematocrit values of 25% to 55%. We studied the possible effect of variations in the volume of sample applied, using several blood and serum specimens. The required sample volume is 30 μL; variations ranging from -2 μL (-7%) to +5 μL (+17%) did not influence the measured value of urate concentration.

Discussion

The good precision of the system was practically the same as recorded with the comparison method. Day-to-day imprecision was particularly low, a result of the reliability of the calibration procedure.

Linearity, a prerequisite for accuracy (11), was very good in the analytical range tested (28–139 mg/L). We did not investigate values beyond this range because genuine serum specimens with such values were not available. The manufacturer claims a measuring range from 20 to 200 mg/L, which roughly corresponds to the concentration range of the calibration material.

Results for serum and whole-blood results agreed favorably with the comparison method values (serum), as also did results for capillary whole blood and venous whole blood (both by the Reflotron).

The uricase/ultraviolet method developed at the Centers for Disease Control (12) might have been a better comparison method, but it was not available at the time of our evaluation. However, subsequent comparison of our secondary comparison method (Urica-quant) with the protocol of the Centers for Disease Control for a few fresh specimens has revealed no systematic bias, in agreement with previous findings (8, 13).

The interference from bilirubin in concentrations >100 mg/L should be considered in the context of the expected use of the system. In a satellite-laboratory environment, or in the doctor’s office, it seems unlikely that blood specimens from severely icteric patients would be submitted for urate assay.

We conclude that the tested Reflotron system is highly precise, practicable, and accurate for measuring urate in either serum or whole blood.

References


CLIN. CHEM. 34/2, 416–418 (1988)

Immunoassay of Low Concentrations of Albumin in Urine by Latex Particle Counting

C. L. Cambiaso,1 D. Collet-Cassart,1,23 and M. Lievea,2

We describe here a nonisotopic immunoassay, based on particle-counting technology, for the determination of urinary albumin. The assay takes only 35 min and has been fully automated on the IMPACT (Acade Diagnostic Systems, Brussels, Belgium) machine. The system measures albumin within a linear range between 6.25 and 50 mg/l and has a detection limit of 0.4 mg/l. Analytical recoveries at three concentrations ranged between 96% and 102%. Within-run precision ranged from 1.6% to 9.5%. The method was compared with a commercial nephelometric immunoassay system and a correlation coefficient of 0.996 was found for 216 urine samples. No antigen excess affects the shape of the curve in our system, whereas in nephelometry a 3 g/l solution of albumin starts to decrease the dose–response curve.

There is an increasing need for albumin measurement more sensitive than that with conventional dip-stick tests. Indeed, albumin excretion up to 20 times greater than normal is not detected by conventional dip-stick tests (7); moreover, the determination of low concentrations of albumin in diabetics is now considered very important for predicting later development of diabetic nephropathy (2, 3).

Assays of low albumin concentrations by immunodiffusion (4), electrommunoassay (5), and radioimmunoassay (6) have been reported. Recently, enzyme immunoassay (7) and fluorimmmunoassay (8) were described for this. The principle of the immunoassay by particle counting as presented here is based on the agglutination of latex particles, covally coated with human albumin, by anti-albumin antiserum and rheumatoid factor as second antibody. To inhibit agglutination, we pre-incubate the sample with the antiserum. The extent of the inhibition reaction is measured by electro-optical counting of residual nonagglutinated particles. In the present system, the albumin concentration is directly proportional to the number of nonagglutinated particles, recorded as peak height. Immunoassays by particle counting have been extensively reviewed elsewhere (9).

Materials and Methods

Materials

Reagents. Human albumin and transferrin were obtained from Behringwerke AG, Marburg/Lahn, F.R.G.; gelatin powder and glycine from Merck, Darmstadt, F.R.G.; and Estapor K150 0.8-μm-diameter latex particles from Rhone-Poulenc, Courbevoie, France. Ultrogel AcA25 was from LKB, Bromma, Sweden.

Buffers. Glycine-buffered saline (GBS) consisted, per liter, of 0.1 mol of glycine, 0.17 mol of NaCl, and 40 mg of NaN3, adjusted to pH 9.2 with NaOH.4 GBS-GT was glycine-buffered saline containing 5 g of gelatin powder and 200 μl of Tween 20 (Technicon Instruments Corp., Tarrytown, NY 10591) per liter.

Anti-human albumin antiserum. A goat was immunized by monthly injections of 100 μg of human albumin in complete Freund's adjuvant. After three months, blood was collected and tested for specific anti-albumin activity by immunoelctrophoresis. Each month, the goat was given booster injections with 100 μg of albumin and was bled 10 days later. We pooled the bleedings for use in the present study.

Rheumatoid factor (RF). We used whole serum from a rheumatoid patient who was undergoing plasmapheresis, with a latex agglutination titer of 1/1250 (determined with latex–rheumatoid factor reagent; Behringwerke AG).

Standards and samples. Human albumin was dissolved in GBS-GT buffer at a concentration of 1 g/l. We diluted this stock solution with the same buffer to obtain standards at concentrations of 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 mg/l.

Urine samples were collected from the clinical department of St-Luc University Hospital and stored frozen before assay. After thawing, the urines were centrifuged and the

1 Unit of Experimental Medicine, International Institute of Cellular and Molecular Pathology, 75, avenue Hippocrate, B-1200 Brussels, Belgium.
2 Unité de Biochimie Médicale, Clinique Universitaire St-Luc, 10, avenue Hippocrate, B-1200 Brussels, Belgium.
3 To whom correspondence should be addressed.

Received September 8, 1987; accepted November 30, 1987.

4 Nonstandard abbreviations: GBS, glycine-buffered saline; GBS-GT, GBS buffer containing gelatin and Tween 20; RF, rheumatoid factor.