Convenient Urine Oxalate Assay with an Oxalate Oxidase Reagent and a Centrifugal Analyzer, Eric Strauss, Matthew Merkin, and James Moishides (Dept. of Clin. Chem., Prince of Wales Hosp., Randwick, N.S.W., Australia)

The urinary oxalate assay described by Laker et al. (1) has been modified and adapted for use with a Cobas Bio centrifugal analyzer (Roche Products Pty. Ltd., Sydney) as an equilibrium method. We use ascorbate oxidase (EC 1.10.3.3), instead of charcoal, to remove ascorbic acid interference. Run on the Cobas Bio, the modified assay is simple, precise, inexpensive, and capable of processing large numbers of patient's urines quickly and conveniently.

Acidify patient's urine to pH <2 with 5 mol/L HCl, then let it stand for 24 h at room temperature to dissolve all oxalate. Centrifuge 25 mL of the specimen, then pass 9 mL of the supernatant through a prepared ion-exchange column (Chelex™ 100 and AG50W-X4, 200–400 mesh resin; Bio-Rad, Richmond, CA) as described (1); discard the effluent, because it will be diluted with column equilibrating solution (0.1 mol/L HCl). Apply a further 1 mL of supernate to the column and save this effluent for oxalate analysis.

To prepare the color reagent, we mixed 1.25 mL of 3-methyl-2-benzothiazolinone hydrazide HCl solution (MBTH; E. Merck, Darmstadt, F.R.G.), 1 g/L in 0.1 mol/L HCl; 2.5 mL of N,N-dimethylaniline/HCl solution (DMA, "Analar grade", BDH, London, England); 2.5 mL/L in 0.1 mol/L HCl; 0.5 mL of peroxidase (EC 1.11.1.7) suspension (Boehringer-Mannheim, Mannheim, F.R.G.), 2.5 kU/L; and 50 mL of potassium citrate buffer (50 mol/L, pH 4.0). For 30 assays, we mixed 10 mL of this color reagent with 0.1 mL of ascorbate oxidase solution (Boehringer-Mannheim; 1700 kU/L) and placed the mixture in the main well of the reagent boat. An oxalate oxidase solution (Boehringer-Mannheim; EC 1.2.3.4, from barley seedlings; 750 U/L) was placed in the start well. Oxalic acid standards were prepared in 0.1 mol/L HCl from analytical grade oxalic acid dihydrate (Baker Chemical Co., Phillipsburg, NJ). Concentrations of 0.2, 0.5, and 1.0 mmol/L were placed in standard wells 1, 2, and 3, respectively. Settings for the Cobas Bio analyzer were: units, mmol/L; calculation factor, 0; standard 1 concn., 0.2; standard 2 concn., 0.5; standard 3 concn., 1.0; limit (mmol/L), 1.5; temperature °C, 30.0; type of analysis, 6; wavelength (nm), 595; sample vol (μL), 8; diluent vol (μL), 15; reagent vol (μL), 300; incubation time(s), 300; start reagent vol (μL), 40; time of first reading (s), 0.5; time interval (s), 900; no. of readings, 5; blanking mode, 1; printout mode, 1.

Replicate analyses (n = 23 days) of a urine yielded a mean oxalate concentration of 0.82 mmol/L (SD = 0.031, CV = 3.84%). The standard curve was linear to at least 1.5 mol of oxalate per liter of urine. Analytical recoveries of oxalate added to urine to give concentrations of 0.5, 1.0, and 1.5 mmol/L (measured in the absence of any added oxalate) ranged from 95.8 to 99.3%. Concentrations of urinary ascorbate up to 5.8 mmol/L did not interfere with the method. The mean oxalate concentration in urine from 28 healthy adults was 0.35 mmol/L (SD 0.075), similar to that reported for an oxalate decarboxylase method (0.37 mmol/L, SD 0.100) (2).

The method has long been used in this laboratory without any problems and serves other pathology centers, which previously used laborious and time-consuming methods. Its performance in the external quality-assurance program operated jointly by the Royal College of Pathologists of Australia and the Australian Association of Clinical Biochemists has been excellent. An analysis of the returned performance reports of this urine quality-control program indicated good correlation (r = 0.9849) between the assigned oxalate concentrations in the survey material and the values measured by the present method. The survey samples, lyophilized human urines prepared by the Commonwealth Serum Laboratories, Victoria, Australia, according to the specifications of the survey organizers, were assigned target values based on the use of a gas-chromatographic method (3). For 27 consecutive specimens with assigned values ranging from 0.08 to 1.50 mmol/L, the correlation coefficient was 0.9849 and the linear regression curve was y = 0.93x + 0.016 (y = present method, x = assigned values). The difference between the assigned values and the values obtained was not significant (P >0.10, paired t-test).

We believe that performing the oxalate oxidase method on the Cobas Bio offers significant advantages over the manual method (1) from which it was adapted.

References

Fat Agglutination Slide Test for Increased Concentrations of C-Reactive Protein in Serum Compared with Results by Laser Nephelometry, Mark Fuller (Dept. of Immunology, Leicester Royal Infirmary, Leicester LE1 5WW, U.K.), Geoffrey Hulman (Dept. of Histopathology, St. George's Hospital, London), and Andrew Macintyre (Dept. of Biology, Liverpool Polytechnic, Byron Street, Liverpool)

The Intralipid® (Kabi-Vitrum Ltd.) fat-emulsion-agglutination test for rapid detection of increases in C-reactive