of a yellow Fe\(^{3+}\)–citrate complex [calibration curve (λ = 390 nm) was linear from 52.9 to 696.6 \(\mu\)g/L, \(n = 8, r = 1.000;\) SD = 0.9; \(F = 26222.44;\) average relative error 0.3%). Phosphate, which can interfere, is eliminated by precipitation.

The usual procedure is as follows: To 4 mL of sample, add 0.1 mL of \(\text{NH}_4\text{OH}\) (30%) and 0.9 mL of \(\text{Mg}^{2+}\) (0.2 mol/L) and filter. Then adjust the pH to 2 (0.1 mL of 10 mol/L \(\text{HCl}\)) and add 0.25 mL of \(\text{Fe}^{3+}\) solution (\(\text{FeCl}_3 \cdot 6\text{H}_2\text{O}, 18 \text{ mmol/L in HCl, 1 mL}\). Read against an iron blank solution (0.25 mL of iron solution in 4.75 mL of HCl, pH 2). Prepare a urine blank by adding 4.75 mL of HCl solution (pH 2) to 0.25 mL of urine (fused of phosphate) and read against pH 2 HCl solution. Subtract the absorbance of the urine blank from the absorbance of the urine sample. Citrate concentration is obtained from a calibration graph [reproducibility (n = 8) was 1.9%; recovery (n = 7) was 96 ± 6%]. Carbohydrates and amino acids do not interfere.

Concentrations of isocitric, malic, and glyoxylic acids (10 times larger), oxalic acid (two times) and salicylic acid larger than those ever found in urine do not interfere. Lactic acid (present in urine only after great exertion) in the maximal concentration ever seen (6.66 mmol/L) causes a 9% increase in apparent citrate.

We compared this method with the citrate lyase "enzymatic" method (Welshman SG, et al., Clin Chim Acta 1973:46:243–6). Regression analysis (n = 18: seven patients with different lithiasis disorders, six normal people, a rat, and four synthetic urine samples) gave \(r = 0.977, SD = 1.07, F = 352.9\).

Values for normal people (936, SD 170 mmol/L) were much higher than those for stone-formers (351, SD 156 mmol/L).

**Immunoturbidimetry of Transthyretin (Prealbumin) in Human Serum, Thomas B. Ledue,¹ Nader Rifai,² Glenn R. Irish,¹ and Lawrence M. Silverman*¹** (¹ Dept. of Product Applications, Atlantic Antibodies, 10 Nonesuch Road, Scarborough, ME 04074; ² Depts. of Pathol. and Hospital Labs., Univ. of North Carolina School of Medicine and North Carolina Memorial Hosp., 1071 Patient Support Tower, Chapel Hill, NC 27514)

Estimates that nearly 50% of hospitalized patients may be malnourished (1, 2) emphasize the need to monitor nutritional therapy. With a biological half-life of 1.9 d, transthyretin (prealbumin) is a sensitive biochemical marker for monitoring patients receiving total parenteral nutrition (3).

We have developed a turbidimetric immunoassay for measurement of serum transthyretin in the Cobas-Bio (Roche Analytical Instruments Inc., Nutley, NJ 07110). Calibrator for transthyretin (Atlantic Antibodies, Scarborough, ME 04074) was diluted in phosphate-buffered (pH 7.4) saline (PBS; per liter, 9 g of NaCl, 8 mmol of \(\text{Na}_2\text{HPO}_4\), 1.5 mmol of \(\text{KH}_2\text{PO}_4\), and 1 g of sodium azide) to prepare a six-concentration curve, range 27 to 430 mg/L. A threefold dilution of goat antiserum to human transthyretin (Atlantic Antibodies) in Polyethylene Glycol 8000 (40 g/L in PBS) was incubated at room temperature for 30 min, then filtered through a 0.45-µm filter before analysis.

Microvolumes of calibrator or patients' sera (4 µL) and PEG-PBS (200 µL) were pipetted into the cuvette rotor. After a 180-s incubation at 25 °C, sample-blank absorbance was measured at 340 nm and 75 µL of diluted antiserum was added. The sample-blank absorbance was then subtracted from the final absorbance, measured 480 s later. Concentrations for samples were calculated with the Cobas "pree" program or from a manual log-log plot of concentration vs absorbance change.

Within-run precision (CV) ranged from 0.9 to 2.0% (n = 20) and day-to-day precision from 1.5 to 3.0% (n = 20) over the concentration range 43 to 412 mg/L. Added hemoglobin (up to 5 g/L) or bilirubin (up to 0.15 g/L) did not interfere.

Sera from 69 subjects were assayed by the proposed method (y) and by rate nephelometry (x; Beckman Auto ICS, Beckman Instruments Inc., Brea, CA 92621). Transthyretin measurements ranged from 30 to 449 mg/L. The linear regression equation for the data was: \(y = 0.99x + 7.4\) mg/L (\(r = 0.988, \text{SEE} = 10.2\) mg/L). Serum samples from 50 healthy individuals (24 men and 26 women) were assayed to establish a reference interval (mean ±2 SD) of 178 to 346 mg/L (mean = 262 mg/L).

With this procedure, quantification of transthyretin is fast (>100 samples per hour), precise, and accurate. The relative ease with which the method can be performed increases its potential as a routine clinical assay of nutritional status.

**References**


**Immunoturbidimetry of Albumin in Serum, Cerebrospinal Fluid, and Urine with a Unique Calibration Curve, Lucile Gerbaut (Lab. de Biochim., Hôpital St Vincent de Paul, 74 Av. Denfert Rochereau, Paris, France)**

We routinely measure albumin in serum, cerebrospinal fluid (CSF), and urine with a Cobas-Bio (Roche Analytical Instruments, Inc.) centrifugal analyzer.

Human albumin standard solutions are prepared in phosphate-buffered (pH 7.4) saline (PBS), with a calibrator (OSAU) from Behring, F.R.G. Dilutions from 1/80 to 1/1280 are prepared in glass tubes. Goat anti-human albumin from Atlantic Antibodies (cat. no. 001-11) is used undiluted. Serum samples are diluted 100-fold in PBS before analysis. CSF and urine protein concentration is first measured with the Coomassie-SDS reagent (I). Samples with a concentration >1 g/L are diluted in PBS before assay.

We use the following parameters: Units (g/L); Calculation factor 1000; Standards 1.7, 3.4, 6.8, 13.6, 27.2, 54.5 mg/dL; Temperature 30 °C; Type of analysis 7.6; Wavelength 340 nm; Sample volume 4 mL; Diluent volume 50 mL; Reagent volume (Polyethylene Glycol 6000, 45 g/L in PBS) 240 µL; Incubation time 60 s; Start reagent volume (undiluted antiserum) 6 µL; Time of first reading 0.5 s; Time interval 30 s; No. readings 10; Blanking mode 1; Calculation mode 1.

Results for serum samples are reported in grams per liter. Results for undiluted CSF and urine samples are multiplied by a factor of 10 and results are thus expressed in milligrams per liter. The concentration range measured in this assay is 17 to 545 mg of albumin per liter. Urine samples with an albumin concentration <17 mg/L are re-analyzed,