of a yellow Fe$^{3+}$--citrate complex [calibration curve ($\lambda = 390$ nm) was linear from 52.9 to 696.6 $\mu$mol/L, $n = 8$, $r = 1.000$; $SD = 0.9$; $F = 263224.4$; average relative error 0.3%]. Phosphate, which 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 HCl) and add 0.25 mL of $\text{Fe}^{3+}$ solution ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 18 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 (freed 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/mL) 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/mL) were much higher than those for stone-formers (351, SD 156 mmol/mL).

**Immunoturbidimetry of Transthyretin (Prealbumin) in Human Serum, Thomas B. Ledue,1 Nader Rifai,2 Glenn R. Irish,1 and Lawrence M. Silvermana,3** (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 Na$_2$HPO$_4$, 1.5 mmol of KH$_2$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-$\mu$m filter before analysis.

Microvolumes of calibrator or patients' sera (4 $\mu$L) and PEG-PBS (200 $\mu$L) were pipetted into the cuvette rotor. After a 180-s incubation at 25 $^\circ$C, sample-blank absorbance was measured at 340 nm and 75 $\mu$L 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 "tubes" 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: $y = 0.98x + 7.4$ mg/L ($r = 0.988$, 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 $^\circ$C; Type of analysis 7.6; Wavelength 340 nm; Sample volume 4 $\mu$L; Diluent volume 50 $\mu$L; Reagent volume (Polyethylene Glycol 6000, 45 g/L in PBS) 240 $\mu$L; Incubation time 60 s; Start reagent volume (undiluted antiserum) 6 $\mu$L; 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.
with the following parameter changes: Sample volume 40 μL; Diluent volume 14 μL; Calculation mode 0. When modified as above, results are reported in milligrams per liter and cover the range 1.7–54.5 mg of albumin per liter.

We assessed the precision of this method by multiple analyses of serum (n = 24), CSF (n = 25), and urine (n = 25) containing 43 g/L, 130 mg/L, and 7.5 mg/L, respectively. Respective intra-assay CVs were 2.9, 1.95, and 4.3%.

Ten urine samples from diabetic children were assayed by the Cobas-Bio and Behring Laser Nephelometer methods. The regression equation relating the Cobas-Bio (x) and nephelometric (y) results was: y = 1.07x − 0.17 (r = 0.998). Albumin excretion by nine healthy adults was 3.2 to 13.3 mg/L, measured in a daytime specimen. This fast, inexpensive procedure avoids problems associated with use of calibration solutions having a low concentration of albumin.

Reference

A Quality-Control System for the “Activated Clotting Time” Test, Frank A. Sedor, Elizabeth Mayo, and Kathryn E. Kirvan (Hospital Laboratories, Dept. Of Pathol, Duke Univ. Med. Center, Durham, NC)

“Activated clotting time” (ACT) is used to monitor heparinization in hemodialysis and cardiopulmonary-bypass surgery, and before arteriography. Rapid (<10 min) and convenient, it can be performed at the bedside and is recommended for monitoring heparinization for patients with no underlying coagulation problems (1, 2). In our hospital, the assay is performed adjacent to the patient, regardless of clinical service, with use of the “Hemochron 400 Heparin Monitoring System” (International Technidyne, Edison, NJ 08820). Arterial blood (2.0 mL) is transferred by air-tight syringe to a Vacutainer Tube containing diastomaceous earth activator and a magnetic stirbar. The timer is started and the tube is inserted into the instrument, activating a motor to drive the stirrer. The act of clotting stops the magnet, triggering an alarm and stopping the timer to mark the ACT.

The laboratory must provide a measure of the reliability of the system. A whole-blood quality-control system is the most desirable but least feasible. Alternatively, the manufacturer suggests a procedure involving activated partial thromboplastin time (APTT) controls at two levels. This procedure requires three pipetting and incubation steps to yield ACTs of approximately 60 and 180 s. This is compared to a target ACT of 400 s for cardiopulmonary-bypass surgery (2), 180 to 240 s for prolonged extracorporeal circulation (3).

We devised a system based on use of outdated fresh frozen plasma (FFP). Titration of 2.0-mL aliquots of FFP with conveniently available CaCl₂ solution (2.72 mol/L, injectable) demonstrated a nonlinear positive cloting response to concentration of calcium. For convenience of preparation and reasonable clotting times, we used 25 μL of the CaCl₂ solution (68 μmol CaCl₂), as follows: (a) Transfer 25 μL of CaCl₂ solution to individual ACT tubes and refrigerate. (b) Mix units of outdated FFP well, and transfer 2.5-mL aliquots into tubes and maintain at −20 °C. As needed, thaw the FFP fractions at room temperature. Initiate the test by pipetting 2.0 mL of FFP into the calcium-adulterated ACT tube, and treat it as if it were a patient’s sample.

The precision of the FFP and APTT systems is tabulated below:

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<tr>
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<th>FFP</th>
<th>APTT</th>
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<tr>
<td></td>
<td>x</td>
<td>CV, %</td>
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<td>6.1</td>
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<tr>
<td>Day to day (n = 20)</td>
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<td>6.8</td>
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Each batch of FFP is usable for at least 21 days. ACT has averaged 261 s during the 18 months the FFP quality-control system has been used. Note that this system addresses only the operation of the instrument. Compared with the commercial APTT reagent, we find the FFP similar in quality as well as economical and convenient.

References

Re-Use of Random Access Fluid Recovered from the Waste of the Technicon RA-1000, Stephen P. Harrison (Dept. of Biochem., Bradford Royal Infirmary, Duckworth Lane, Bradford, BD9 6RJ, U.K.)

“Random Access Fluid” (RAF), a polyfluorinated hydrocarbon liquid, is used in the RA-1000 to eliminate carryover between samples and reagents. We use about 4 L per annum and, at £0.50 per milliliter, this contributes significantly to the running costs for the instrument. Because RAF is an inert material, immiscible with water (1), most of it may easily be recovered and reprocessed, to be used again. RAF is deposited at four sites in the RA-1000: in the reaction tray, in the reagent boats, in the sample- and reagent-probe reservoirs, and in the sample cups. RAF deposited in the sample cups is potentially dangerous to recover, but the waste from the other three sites may be collected into a suitable wide-mouthed container half filled with a sterilizing 50 g/L solution of sodium hypochlorite and reprocessed, conveniently at monthly intervals, as described below.

The waste liquid collected consists of two phases: the top aqueous layer and the lower RAF-reagent precipitate–water emulsion layer. Most of the aqueous layer is removed by pouring off and the lower layer is washed repeatedly with water, pouring off after each addition, until most of the reagent precipitate is removed. The unrefined RAF is transferred to a separatory funnel, where the lower RAF emulsion is removed, leaving any remaining reagent precipitate in the funnel. The RAF is further sterilized by mixing with an equal volume of 50 g/L sodium hypochlorite solution. The layers are allowed to separate and the upper aqueous layer is removed. The RAF emulsion is now washed in a similar manner with three equal volumes of distilled water. After the final wash as much as possible of the top (aqueous) layer is removed and the emulsion is dehydrated by adding anhydrous sodium sulfate (about 1 g per 5 mL of RAF). The