Three Immunoassay Methods Evaluated for Quantifying Prealbumin (Transhyretin) in Serum

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We developed an immunoturbidimetric assay for prealbumin on the Cobas Bio centrifugal analyzer and compared results from this assay with those from a rate nephelometric assay (Beckman Instruments Inc.) and a radial immunodiffusion kit (Behring Diagnostics). All three assays were evaluated for precision, linearity, and correlation to each other for analysis of sera from pediatric patients. All assays gave similar results for patients' samples. Values were higher by the radial immunodiffusion assay than by the other two methods, which gave similar results for the same specimens. We conclude that the immunoturbidimetric and rate nephelometric assay for prealbumin are acceptable alternatives for quantifying prealbumin in serum and also have a faster turnaround time than radial immunodiffusion.

Additional Keyphrases: immunoturbidimetry • radial immunodiffusion • rate nephelometry • pediatric chemistry • reference interval

Immunoassays for prealbumin (transhyretin) have been used primarily to assess patients with liver disease (1, 2) or protein-calorie malnutrition (3). For many years, the only commercial kit available for quantifying serum prealbumin was Behring's radial immunodiffusion (RID) assay.2 However, several manufacturers have recently marketed immunoassay kits for prealbumin with faster turnaround times than the RID assays.

Here we describe an immunoturbidimetric (IT) assay for prealbumin on the Cobas-Bio centrifugal analyzer. We evaluate this assay, a rate nephelometric assay (Beckman ICS), and the Behring RID assay for precision and linearity. In addition we compare results of these assays for serum from a pediatric patient population. All three assays gave similar results in these studies, but the ICS and IT methods had a faster turnaround time and superior precision and linearity.

Materials and Methods

The three immunoassays we evaluated were a RID assay (Behring Diagnostics, La Jolla, CA); an ICS assay (Beckman Instruments Inc., Brea, CA), and an IT assay that we developed for the Cobas Bio centrifugal analyzer (Roche Analytical Instruments Inc., Nutley, NJ) equipped with the dens option (version 8326C).

The ICS assay was a pre-production reagent kit with a rabbit antiserum to prealbumin and was performed as described previously (4) in an Auto ICS equipped with an Accu-prep automatic pipet (Model 222A; Beckman Instruments Inc.). Initially, we used a 10-fold dilution of serum to assay concentrations between 50 to 400 mg/L. This range can be extended by using a six- or 216-fold dilution of serum for analysis. However, for six-fold-diluted serum samples we used ICS Buffer (described in ref. 4) as diluent, centrifuged (8000 × g, 5 min) the samples at room temperature, and assayed the supernates.

In the IT assay, we used goat antiserum to human prealbumin (lot 011; Atlantic Antibodies, Scarborough, ME), diluted 40-fold in modified ICS Buffer (one part deionized water and nine parts ICS Buffer) and allowed to stand for at least 1 h at room temperature before filtration through a 0.2-μm (sw. pore size) filter (Gelman Science Inc., Ann Arbor, MI). The filtered antiserum was placed in the primary reagent well of the reagent tray of the Cobas Bio. Calibrators (Behring Diagnostics; Standard Human Serum, lot 1015D (or E) and 11715 with prealbumin concentrations of 370 and 340 mg/L, respectively), controls, and patients' samples were diluted in ICS Buffer immediately before assay. Patients' sera were initially diluted 12-fold in ICS Buffer; all samples were centrifuged (8000 × g, 5 min) at room temperature. The supernatant fluid was then transferred to a Cobas Bio sample cup, which was placed on the sample disc of the Cobas Bio.

The instrument settings we used for the Cobas Bio were identical to those defined by Foster and Leduc (5) for the auto blanking method. The volumes of sample, diluent, and
reagent were 20, 10, and 200 μL, respectively. The concentrations of calibrators were 50, 100, 150, 200, 300, and 400 mg/L. Data from the six calibrators were used to construct a calibration curve, and the instrument printed out the results for controls and patients' samples. If the concentrations of prealbumin were <50 or >400 mg/L, the samples were diluted six-fold and 18-fold, respectively; processed; and reanalyzed as described above.

For precision studies we used ICS control serum (lot no. 411200; Beckman), a pooled human serum that contains about 290 mg of prealbumin per liter. We further diluted this serum with normal rabbit serum or normal goat serum to obtain prealbumin concentrations of about 100, 50, and 35 mg/L.

We evaluated the within-run precision of the ICS and IT assays and between-run precision of all three assays. For the former we assayed 20 samples from each control pool; for the latter we used single determinations for each control pool in 10 to 20 separate analyses.

The linearity of the standard curves for the three assays was determined by diluting ICS Calibrator I (lot no. C504013; Beckman Instruments Inc.) with different volumes of normal human serum. The serum dilutions are expressed as the percentage of human serum (by volume) present in the total volume of serum (human and animal). We assayed these dilutions by both the RID method or further diluted them as described above for the ICS and IT assays. In the ICS and IT assays, the volume percent of human serum measured at each assay dilution was as follows: 2, 4, 6, 8, 10% serum assayed at six-fold dilution for the ICS and IT assays; 20, 30, 40, 50, 60, and 70% serum assayed at 12- and 36-fold dilution for ICS and IT assays, respectively; and 80, 90, and 100% serum assayed at 18- and 216-fold dilution for ICS and IT assays, respectively.

The linearity experiments were performed by assaying all human serum dilutions in duplicate. For the RID assay, the standards, controls, and human serum dilutions were set up on the same rotor, and prealbumin concentrations were determined by using a stored calibration curve that was generated in the run just preceding the assay.

Correlation studies of patients' results were performed with leftover serum samples from a pediatric patient population (114 sera) or from normal adults (11 sera). Sixty-six of the pediatric sera came from 16 nutritionally compromised patients; 13 of these 16 patients were less than one year old and were hospitalized in a neonatal intensive-care unit.

Data from the linearity experiments and correlation studies with patients' samples were analyzed by standard linear regression analysis and modified linear regression analysis (6), respectively. The latter analysis assumes error in both variables. The data from the patients' studies were further analyzed by Student's paired-t test.

Results

Development of an IT assay on the Cobas Bio centrifugal analyzer. In initial experiments we determined the range of concentrations of prealbumin in the calibration curve, the dilution of antisera, and the volume of sample used in the IT assay. Assay of 10- or 20-μL serum volumes, a series of different concentrations of prealbumin, and different dilutions of antisera showed the best results were obtained when we used 20 μL of sample and a 40-fold diluted antisera with sera containing 50 to 400 mg of prealbumin per liter. Figure 1 presents a representative calibration curve for the IT assay. Assay of Atlantic Antibodies calibrator I (lot 230; prealbumin 750 mg/L) showed that antigen excess was still present with prealbumin concentrations as great as 894 mg/L.

Evaluation of the immunoassays.3 The precision of the assays is summarized in Table 1. Both ICS and IT assays gave similar within-run and between-run precision. In contrast, the RID assay gave higher between-run CVs than those obtained by the ICS and IT assays.

The analytical range of an assay, the range of concentrations that can be accurately detected without modification to the assay, was 35 to 470 mg/L for the RID assay; for the IT and ICS assays, the analytical range was 50 to 400 mg/L. Linear-regression analysis of the data from the linearity experiments showed that, within the analytical range of the ICS and IT assays, the linearity was improved when samples with concentrations between 50 and 60 mg/L were assayed at a six-fold dilution (both assays) and when samples with concentrations >350 mg/L were assayed at a 18-fold dilution (IT assay). Overall, the ICS assay was superior to the other assays in showing minimal scatter about the regression line: for ICS, y = 5.23x + 2.04 mg/L, r = 1.0, S_x = 0.82 mg/L, n = 28; for IT, y = 4.70x + 17.04 mg/L, r = 1.0, S_x = 0.98 mg/L, n = 26; and for RID, y = 5.02x + 19.40 mg/L, r = 0.99, S_x = 1.57 mg/L, n = 12. Moreover, the standard curve for the ICS assay showed better linearity than the one for the IT assay at concentrations between 10 and 50 mg/L. Beckman has modified the stated analytical range on the production lot of the ICS kit to be 70–500 mg/L.

We found good correlation between the prealbumin results obtained with all three assay methods after analysis of the patients' samples: ICS = 0.84RID + 5.5 mg/L, r = 0.92, S_x = 3.72 mg/L, n = 116; IT = 0.77RID + 27.52 mg/L, r = 0.93, S_x = 3.32 mg/L, n = 108; ICS = 1.04IT - 23.67 mg/L, r = 0.94, S_x = 3.34 mg/L, n = 113. Statistical analysis by a Student's paired-t test showed that the inter-test differences...
were statistically significant: for RID vs ICS, $P < 0.0001$; for RID vs IT, $P < 0.0001$; and for IT vs ICS, $P = 0.0186$ (the corresponding 95% confidence intervals for mean differences between values were 1.84 to 3.44, 1.15 to 2.84, and 0.12 to 1.31, respectively). The differences between the RID and the ICS or IT results were greater in serum samples in which prealbumin concentrations exceeded 200 mg/L.

**Discussion**

Recently, the increasing interest in using prealbumin as a visceral protein to monitor nutritional therapy (3) has led several companies to manufacture assays for quantifying prealbumin in serum. We have developed an IT assay for the Cobas Bio centrifugal analyzer and compared it with a pre-production Beckman ICS kit and the Behring RID method. The only change from the pre-production ICS kit to the production lot kit was a change in the analytical range, to 70 to 500 mg/L.

We developed the IT assay from information supplied by Beckman Instruments Inc. and Atlantic Antibodies Inc. In 1980, the ICS Applications Group at Beckman Instruments Inc. developed a manual-mode rate nephelometric assay and found that it gave results similar to those obtained by the Behring RID kit. In this assay, rabbit antiserum to human prealbumin was diluted threefold in ICS Diluent serum and was diluted 12-fold in ICS Buffer. A protein standard serum from Behring was used to construct a calibration curve. All serum samples were centrifuged (8000 $\times g$ for 5 min) at room temperature before the supernates were assayed as described above (4). This information, plus that obtained from Atlantic Antibodies on the Cobas Bio instrument parameters and the use of modified ICS buffer to dilute the antiserum, served as the basis for developing the IT assay.

Our results and those obtained earlier (7–9) show that pretreatment of serum by dilution in buffer containing polyethylene glycol and subsequent centrifugation does not remove appreciable amounts of prealbumin from human serum. Calculation of the mean difference between the results showed better agreement between the IT and ICS assays than between the RID and IT or ICS assays. In contrast, Odouka and Ferard (8) found a 14% to 18% increase in serum prealbumin when results for specimens pretreated by diluting in a saline solution containing 40 g of polyethylene glycol per liter, then centrifuging, were compared with those for untreated specimens in the Behring RID assay and in a two-point nephelometric assay. This difference may reflect the nature of their comparison (within-assay rather than between-assay, as in our report), the formulation of the ICS Buffer, and (or) the assay methodology (the two-point nephelometric assay involves a 60-min incubation at room temperature).

Our IT assay differs from the IT assay recently published by Hamlin and Pankowsky (10). Although both methods are used with the Cobas Bio, their assay does not pretreat the serum by centrifugation, but utilizes a serum blanking step before addition of the antiserum and is performed at 37 °C. Comparison of the precision studies for both assays shows similar results.

We found that the ICS and IT assays gave superior between-run precision and less scatter about the regression line in the linearity studies than found by the RID method. The correlation studies with serum specimens from patients whom we would evaluate in our hospital showed good agreement between the results of the IT and ICS assays. However, similar comparison of the RID and ICS or IT results showed that the RID method yielded higher values, especially evident in specimens with prealbumin concentrations exceeding 200 mg/L. This discordance in the results between the Behring RID and ICS methods has also been reported by others (11). This discrepancy may be due to a difference in the methods used to assign values to the calibration material (10). We and Beckman Instruments found approximately 10% lower values than expected when the Behring calibrators were assayed by the ICS method. However, this difference is minimized in our IT assays because we use the Behring calibrator material.

Tables 2 compares different features of the assays we evaluated with those published earlier evaluating the Abbott enzyme immunoassay (EIA) (12). The major advan-

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**Table 1. Precision of ICS, IT, and RID Immunoassays of Prealbumin (mg/L)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Behring RID</th>
<th>Beckman ICS</th>
<th>IT</th>
<th>Abbott EIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen vol per test, µL</td>
<td>5</td>
<td>100 $^*$</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Specimen</td>
<td>Serum/plasma</td>
<td>Serum</td>
<td>Serum/plasma</td>
<td>Serum/plasma</td>
</tr>
<tr>
<td>No. of stds.</td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Analytical range, mg/L</td>
<td>35–70</td>
<td>70–500 $^*$</td>
<td>100–350</td>
<td>50–500</td>
</tr>
<tr>
<td>Turnaround time per sample, h</td>
<td>18 or 48</td>
<td>0.25</td>
<td>0.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Detection limit, mg/L</td>
<td>35 $^a$</td>
<td>11 $^a$</td>
<td>28 $^a$</td>
<td>1 $^d$</td>
</tr>
</tbody>
</table>

$^*$Based on kit insert supplied by manufacturer and on reported evaluation (12).

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Based as little as 20 µL can be used with the Array Protein Analyzer.

$^*$Production lot of Beckman ICS kit.

$^a$Based on data presented in kit insert.

$^*Lowest detectable limit from linearity experiments.
Rapid Typing of Serum Paraproteins by Immunoblotting without Antigen-Excess Artifacts

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In this new immunoblotting procedure for determining the heavy-chain class and light-chain type of monoclonal serum immunoglobulins, proteins are transferred from agarose electrophoretic gels to nitrocellulose by brief capillary blotting. Paraproteins transferred are detected with appropriate horseradish peroxidase-conjugated antisera to light chain and heavy chain. Examination of 121 serum specimens probably containing a paraprotein (as detected by protein staining) by immunoblotting and by immunofixation gave the same results for 116 specimens: paraproteins were typed in 103 specimens and their presence was excluded in 13. Immunofixation required one repeat analysis (as compared with 22 for immunoblotting). In only 100 min we could type as many as 10 paraproteins, and the procedure did not show antigen-excess artifacts. These results suggest that immunoblotting may be preferable to immunofixation for routine typing of paraproteins.

Additional Keyphrases: enzyme-conjugated antisera • monoclonal immunoglobulins • electrophoresis, agarose • isoelectric focusing

Determination of the heavy-chain class and light-chain type of a serum paraprotein may give important diagnostic and prognostic information (1–3). Immunochemical demonstration of only one light-chain type confirms the presence of a paraprotein, a situation that may have been suspected from protein staining. The finding of a paraprotein in low concentration can be crucial early in a myeloproliferative disease (4). Immunoglobulin typing is also required to identify the \(\mu\)-heavy chain of Waldenström's macroglobulinemia; the \(\delta\)-heavy chain of IgD myeloma, with its poor prognosis; free light chains associated with renal failure; and biclonal immunoglobulins (1, 2).

Immunofixation after electrophoresis appears to be the best currently used routine method for paraprotein typing (2, 3, 5), but it has several disadvantages. Antigen-excess artifacts may require repeat examination of specimens at several dilutions of serum or antisem (2, 3); these effects distort or even obscure the precipitation required for analysis. Immunofixation also requires considerable technical expertise and experience in interpretation. It is time consuming, often including overnight-washing steps (2, 6).

Here we describe a simple, rapid immunoblotting method and compare its performance with that of immunofixation for typing serum paraproteins in the routine laboratory. Immunoblotting is more sensitive than immunofixation, less prone to artifacts that cause false results, and is easier to interpret.

Materials and Methods

Nitrocellulose sheet, 0.45-\(\mu\)m pore size (Schleicher & Schuell, type BA85), was supplied by Anderman & Co. Ltd.

References