Development and validation of an automated latex-enhanced immunoassay for prealbumin

Peter Holownia,∗ David J. Newman, Hansa Thakkar, William D. Bedzyk, Helen Crane, Yemi Olabiran, Carol L. Davey, and Christopher P. Price∗

The measurement of circulating prealbumin has been shown to be clinically useful in the assessment of nutritional status, both as an initial screen and in the monitoring of nutritional recovery. We describe a fully automated, noncompetitive, homogenous, light-scattering immunoassay that has been developed for this analyte on a Dimension® (Dade) analyzer. A sheep anti-prealbumin IgG fraction was covalently coupled to 40-nm chloromethyl styrene particles and, after the addition of sample, polyethylene glycol-assisted immunoonagglutination was monitored by turbidimetry. The prealbumin working assay range was 8–550 mg/L at a sample volume of 2 μL and a reaction time of 6.5 min. When data were analyzed using ANOVA, total and within-run assay imprecision values (CVs) were 1–5%, and calibration and reagent stabilities were in excess of 40 days. Mean analytical recoveries were 102% ± 4% (SD), and there was no lack of parallelism. Hemolysis, lipemia, and bilirubin did not interfere. Both plasma anticoagulated with heparin or EDTA and serum from plain or serum-separation tubes were acceptable as sample matrices. Comparison with the Beckman Array® method gave a Passing and Bablok regression of: Dimension analyzer = 1.01Beckman + 7.1 (n = 103), using a common calibrator. We conclude that the prealbumin method is appropriate for clinical use according to the analytical criteria used in this study.

Adequate nutrition is now recognized as important for hospital patients during illness, recovery, and treatment (1–4), substantially improving mortality and morbidity rates (5–7) and decreasing the length of hospital stays (4, 7). Protein calorie malnutrition, which consists of a range of pathologies that arise in varying degrees from a lack of protein and calories, is known to impair wound healing, immunocompetence, and muscle and neuronal function (1, 8). Occult protein calorie malnutrition is widespread in many hospitalized patients throughout the world (1, 4, 5), making the development of an adequate nutritional strategy an important issue in healthcare. Assessments of nutritional status usually (1) include anthropometric tests of body composition (protein-energy reserves), investigation of patient history, and clinical examination (e.g., muscle wasting or changes in body weight unrelated to body water). In addition, biochemical tests are used to assess protein energy status by measurement of serum proteins or urinary excretion of nitrogen. Each of these approaches has attendant problems (7), consisting of, in various degrees, nonspecificity, unreliability of measurement, and slow response to nutritional status.

At present there is little general agreement on a single test or panel of laboratory tests that may serve as a definitive means of monitoring nutritional status (1–19). The proposed criteria of an “ideal” serum marker of nutritional status (8) are, in summary, a short biological half-life and a specific and fast response to protein/energy status. Circulating proteins and their half-lives that reflect protein/energy homeostasis (5, 7, 12, 20) include albumin (≈20 days), transferrin (≈8.5 days), prealbumin (≈2 days), fibronectin (≈4–24 h), and retinol-binding protein (≈8–12 h). Within this context, prealbumin is the candidate whose characteristics best match the requirements because the intermediate half-life yields the best diagnostic sensitivity and specificity (2, 4, 9, 14). Prealbumin is especially sensitive to early phases of decreased nutrition, particularly caloric intake (1, 2, 7) and nitrogen balance; in addition, the concentration of prealbumin rapidly returns to values within the reference interval once the malnutrition has been corrected (1, 7, 16). For these reasons, the measurement of prealbumin has been shown to be clinically useful in monitoring nutritional status (4, 9–11) and/or nutritional

1 Department of Clinical Biochemistry, St. Bartholomew’s and the Royal London School of Medicine & Dentistry, Turner Street, London E1 2AD, UK.
2 Dade International, Glasgow Site, Wilmington, Inc., Newark, DE 19898.
*Author for correspondence. Fax (44)/71-377-1544; e-mail c.p.price@mds.qmw.ac.uk.

Received October 17, 1997; revision accepted March 3, 1998.
bances at the defined time points and wavelengths (“filter International). The assay data consisted of raw absor-

munoassay; PR, particle reagent; and PEG, polyethylene glycol.

procedures for assay preparation and analysis

-phase reactant, such as C-reactive protein (5, 17, 18). C-re-

active protein has a similar half-life to prealbumin and can be measured using the same technology (19). The physi-

ological role of prealbumin is actually that of a transport protein of thyroid hormones and the vitamin A/retinol-

binding protein complex to target tissue, whereas in the circulation prealbumin exists as a stable tetramer bound to retinol-binding protein (21).

The broad consensus for an adult reference range for prealbumin is 120–500 mg/L, with considerable variation within the quoted ranges depending on the study, the numbers tested, and the methods used (19–27). A small difference between the sexes exists, with the male range being marginally lower than the female range. The range in infants is ~40–150 mg/L (24, 25); however, during childhood and puberty the concentrations for both sexes rise gradually, and at different stages of development, there appear to be some marginal differences between sexes (26, 27). Various studies (4, 13) have suggested that a concentration of <110 mg/L is considered high risk, requiring major nutritional therapy; 110–170 mg/L is moderate risk with less intensive nutritional therapy requirement; and >170 mg/L is of little or no risk. Reference range values of prealbumin, therefore, represent the high range of any assay, whereas a decrease from these concentrations will be of clinical use. Methods available for the measurement of prealbumin are mainly based on immunoprecipitation and include electroimmunoassay (19), immunodiffusion (23), and light-scattering free solution assays (28). The two former techniques are time-consuming, and the conventional nonenhanced light-scattering assays operate at the limit of sensitivity of the assay, requiring very careful choice of antisera. The opportunity to use a particle-enhanced method would provide greater sensitivity and reduce the use of anti-

body. To this end, a latex-particle-enhanced turbidimetric immunoassay (PETIA)\(^3\) was developed to meet the clinical criteria of a wide working range with good precision and specificity.

### Materials and Methods

#### Procedures for Assay Preparation and Analysis

**Instrumental analysis.** The prealbumin method was run on the “Open Channels” software that is an option available on the Dimension® Clinical Chemistry System (Dade International). The assay data consisted of raw absor-

bances at the defined time points and wavelengths (“filter data”), which were captured on computer, using a switching device into a Microsoft Excel\(^6\) spreadsheet via an interface communications application (Versaterm Pro\™, Synergy Software, PCS). In addition, the Dimension “kinetics” software option allows continuous monitoring of reaction progress curves at 5-s intervals over the 10 wavelengths available on the instrument, if needed.

**Preparation of antibody particle reagent (PR).** A polyclonal antibody, IgG fraction, (Binding Site; cat. no. PCO66) was covalently coupled to 40-nm diameter latex particles consisting of a polyvinyl-naphthalene core and a chemi-

cally reactive chloromethyl styrene shell (Dade Interna-

tional) (29). Particles were added in a slow dropwise fashion to the diluted antibody with gentle shaking. The final concentrations of antibody and particles for coupling were 2.5 g/L protein and 5 g/L dry solids, respectively, in 15 mmol/L sodium phosphate buffer, pH 7.5, 0.5 mL/L Gafac RE610 detergent (Gafac, Manchester, UK). The mixture was incubated overnight at 37 °C in an orbital shaker (Infors HT, Crewe, UK) at 240 rpm followed by ultracentrifugation at 40 000g for 60 min at 20 °C. The supernatant was carefully removed and discarded, whereas the pellet was resuspended in wash buffer (50 mmol/L glycine, 0.05 mL/L Gafac, and 0.1 g/L azide, pH 7.5) made up to the volume of the starting reaction mixture. This was followed by three similar wash steps, after which the final pellet was resuspended in a volume of storage buffer (500 mmol/L glycine, 0.5 mL/L Gafac, and 1 g/L azide, pH 7.5) equivalent to one-half of the original volume of the starting coupling reaction mixture. The final preparation of PR was placed in an ice bath and sonicated by two bursts of 30 s duration (20 microns intensity) with an interval of 1 min between bursts, using an MSE Soniprep 150 (Fisons, UK). The PR was now ready for use in the assay; the working dilution was adjusted with 5 mmol/L glycine, 0.005 mL/L Gafac, and 0.01 g/L azide, pH 7.5, such that the absorbance at the active measuring wavelength of 383 nm (termed as “initial absorbance”) was 1000 milliabsorbance units before the addition of sample.

**Coupling reproducibility.** Identical lots of particles and IgG fractions and identical scales of synthesis (volumes of reaction mixture) were used to generate calibration curves from separate syntheses (n = 3) and to determine the variation (CV) in signal at each concentration of prealbumin.

**Calibrators and matrix.** The purified prealbumin used as the calibrator material was provided by Scipac (Kent, UK; product code P171–1, >96% purity). A stock solution of calibrator (1000 mg/L), was prepared in an artificial matrix (HEPES-buffered bovine serum albumin with pres-

ervatives) supplied by Dade International. A zero cali-

brator and a series of five working calibrators were prepared by diluting the stock solution to values assigned

---

\(^3\) Nonstandard abbreviations: PETIA, particle-enhanced turbidimetric immunoassay; PR, particle reagent; and PEG, polyethylene glycol.
by the Beckman Array® and calibrated according to IFCC reference material CRM470 (30).

ASSAY DEVELOPMENT
Within the operation framework of the Dimension analyzer, all selections of materials for reagents and their optimizations were performed to meet specifications of a maximum change in signal over the required assay range, to achieve the best reproducibility of performance and the lowest nonspecific response.

Nonenhanced assay. The alternative approach of using free antibody in place of latex enhancement in the assay produced very poor signal changes of antibody in place of latex enhancement in the assay. The alternative approach of using free Nonenhanced assay. The lowest nonspecific response.

Antibody loading. A binding site antibody was chosen from a variety of commercial and in-house sources, both IgG- and affinity-purified, which best fulfilled the assay criteria. The working range of the assay (0–550 mg/L), was achieved by minimizing the sample volume and using the maximum amount of antibody within the constraints of the system. This was done by using high loadings of antibody and additions of concentrated PR. A 2.5 g/L loading of antibody onto particles was chosen because the higher loadings that had been used previously had produced colloidal instability and steric hindrance. (31).

PR dilution and wavelength selection. A system requirement for the assay was an initial absorbance of ≤1000 milliabsorbance units at the active wavelength of measurement. This ensured that the rise in signal caused by immunoagglutination stayed within the 2000 milliabsorbance-unit upper limit of absorbance linearity (Beer–Lambert Law), as well as the measuring range of the spectrophotometer on board the Dimension system. At any given working dilution of PR, the initial absorbance decreases with increasing wavelength, according to the Rayleigh light-scattering theory when particle sizes are ≈<1/10 of the wavelength of incident light. This provided the means of extending the working range of the assay by increasing the amounts of PR (by altering the volume of added PR) at higher wavelengths while maintaining the initial absorbance at 1000 milliabsorbance units.

Assay buffer. Conditions were optimized such that the enhancing effect of PEG 8000-assisted agglutination, (PEG obtained from Sigma, UK), was offset by maintaining a negative charge on the antibody at pH 7.5, (IgGs have a pI~6.0) and by the addition of an anionic detergent to increase PR stability by decreasing nonspecific binding. The presence of phosphate has also been shown (28) to aid complex formation (antichaotropic agent); therefore, the concentration of phosphate was minimized from the starting conditions of a 150 mmol/L phosphate buffer to 15 mmol/L while maintaining the ionic strength with sodium chloride. This also prevented the potential carryover of phosphate interference into other analytical tests. The final conditions are described in the assay protocol section.

Assay protocol. Reagents were introduced into empty, punctured reagent containers (flexes™) as follows: 4 mL of assay buffer (15 mmol/L sodium phosphate buffer, 135 mmol/L sodium chloride, 14 g/L PEG, and 0.1 mL/L Gafac) was added to wells 1–4, and 2.6 mL of PR was added to wells 5 and 6. The immunoagglutination reaction was started by the addition of 2 μL of sample, from a total sample volume of 30 μL present in the sample cup, after a 100-s preincubation of assay buffer (346 μL) with PR (105 μL). The assay signal was determined from the captured filter data, incorporating a sample blank (i.e., the difference between two bichromatic readings at the active wavelength of 383 nm and reference wavelength of 700 nm), taken 30 and 300 s after sample addition.

Determination of prealbumin result. A calibration curve was constructed using an iterative five-parameter logistic (logit) curve-fitting procedure, as found on the Dimension system, but using a software package (Deltagraph® software; Delta Point) to calculate the initial coefficients off-line. The concentrations of unknown prealbumin samples were determined using the logit algorithm on a Microsoft Excel spreadsheet, where the concentration was calculated from the signal and generated coefficients.

Statistical analyses. Individual aspects of reproducibility, such as assay imprecision, calibration stability, and reagent stability (on board the Dimension analyzer), were assessed by the total reproducibility (interassay variation) and the within-run reproducibility (intraassay variation), using the ANOVA procedure recommended by the NCCLS (32). Using Microsoft Excel, Ver. 5.0, software, a spreadsheet was constructed to perform the ANOVA calculations. A χ² test was used to assess the significance of the calculated reproducibility values relative to chosen values (e.g., CV 5%) or to identify the significance of factors contributing to the total imprecision. The agreement between methods was assessed by Passing and Bablok regression (33), which accounts for errors in both x and y planes, makes no assumption on the error distribution, and in which the estimated line of best fit is not unduly influenced by extreme points or outliers. A Bland–Altman (34) procedure was used to assess bias. The statistical package used was the Astute™ (Diagnostic Development Unit, University of Leeds, UK). Simple linear regression was used to assess parallelism.

EVALUATION AND VALIDATION
Analytical recoveries and parallelism. Recoveries were assessed by the addition of two different amounts of puri-
fied prealbumin (stock 1000 mg/L, weighed into the calibrator) to nine nondiseased serum samples such that the volume of the supplement was <10% of the total sample volume. The recovery with endogenous prealbumin was assessed by experiments where 10 samples containing relatively high prealbumin concentrations (range, 260–400 mg/L range) were each diluted (by factors of 0.2, 0.4, 0.6, or 0.8) with 10 samples containing relatively low concentrations of prealbumin (range, 60–160 mg/L). Parallelism was determined by analyzing 10 serum samples containing prealbumin concentrations in the 250–450 mg/L range that had been serially diluted with saline (×0.25, ×0.5, ×0.75, and ×0.875).

Imprecision. The within-assay and total reproducibility were calculated by ANOVA, giving within-assay and total reproducibility values. A re-analysis of the imprecision data calculated according to the calibration from day 1 was performed to determine the calibration stability. The reagent stability on board the analyzer, with already punctured flexes as required by the Dimension analyzer open channels format, was also assessed from captured filter data over the same occasions and period of time. The results were likewise calculated according to day 1 calibration.

Calibration and reagent stability. Both calibration and reagent stability were analyzed by ANOVA, giving within-assay and total reproducibility values. A re-analysis of the imprecision data calculated according to the calibration from day 1 was performed to determine the calibration stability. The reagent stability on board the analyzer, with already punctured flexes as required by the Dimension analyzer open channels format, was also assessed from captured filter data over the same occasions and period of time. The results were likewise calculated according to day 1 calibration.

Accuracy. The developed PETIA was compared with the selected reference method (Beckman Array system, Beckman Instruments), which used a calibrator assigned with the IFCC reference material CRM 470 (30). Serum specimens that had been kept at 4 °C for <1 week were obtained from the hospital routine clinical laboratory for simultaneous analysis by both methods. Samples were drawn from the following patient groups: reference interferences (n = 41), low albumin (~25 g/L; n = 20), renal patients with urea >15 mmol/L (n = 18), lipemic and icteric (n = 5), and quality controls (n = 19), obtained from Behring (cat. no. ORHA), Incstar (cat. no. SPQ), Bio-Rad (cat. no. 1587) and Dako (cat. no. X94) in the United Kingdom and Fitzgerald (cat. no. 35-PC5) in the United States.

Interference and cross-reaction with albumin. Interference was assessed by the addition of the following interferents at the stated final concentrations in six serum samples containing measured prealbumin concentrations of 80, 103, 118, 140, 180, and 250 mg/L: hemoglobin at 500–7700 mg/L, prepared from washed, hemolized erythrocytes and concentrated by ultrafiltration; bilirubin at 4.4–550 μmol/L, prepared in a stock solution in sodium carbonate/dimethyl sulfoxide; and triglyceride at 1.5–12.2 mmol/L, obtained in the form of Intralipid 20% fat emulsion from Pharmacia. Conditions were arranged such that the volume of added interferents was <5% of the total sample volume.

The effects of commonly used blood sample collection procedures in a routine clinical laboratory environment were also investigated. Blood was obtained from 10 healthy subjects, using serum (plain tubes), serum with SST gel-clot activator, and plasma from Li-heparin and EDTA tubes. Prealbumin was measured during the same day in all samples.

A series of human serum albumin (Behring/Hoechst, UK, cat no. ORHA) samples were prepared in the range 1–10 g/L and measured in the prealbumin assay to assess cross-reactivity.

Assay range and sensitivity. Precision profiles were obtained by curve-fitting a second-order polynomial function to the relationship between the SD and the CV of duplicate measurements, with respect to binned prealbumin concentration from the Dimension analyzer comparison results. In addition, a duplicate analysis was performed on seven samples that contained low concentrations of prealbumin to allow a better definition of the binned range, 50–100 mg/L. Because of insufficient volume, these samples were not available for the method comparison. The assay range was defined as the prealbumin concentration range within which the CV was acceptable to the analyst, generally accepted to be at the CV cutoff of 10% (35). The sensitivity (detection limit) as defined by the error of measurement at zero dose (35) was obtained from the intercept of the SD precision profile.

Sample and calibrator stability. Three serum sample pools and two freshly prepared calibrators were measured after storage under the following conditions: five freeze-thaw cycles during a single day, based on an initial calibration curve, and during days 1, 2, 3, 5, and 10 of incubations at room temperature (~20 °C), 4 °C, and −20 °C.

Results

DETERMINATION OF PREALBUMIN RESULT

A typical calibration curve, prepared under the finalized assay conditions (Fig. 1), was generated using the following coefficients: \( C_0 = -5.5795, C_1 = 248.2, C_2 = -4.679, C_3 = 605.4, \) and \( C_4 = 0.5. \) This was based on the logit curve fit of the form: Absorbance = \( \frac{C_1}{1+((concentration/C_2)+C_4)^{C_3}+1}+C_4. \)
PR DILUTION AND WAVELENGTH SELECTION
An active wavelength of 383 nm was selected; Fig. 2 shows the respective effects, at two different amounts of PR, of increasing the wavelength on the standard curves of the assay and the initial absorbances. The 340 nm wavelength gave working ranges of prealbumin at prohibitively high initial absorbances of >1360 milliabsorbance units, whereas the higher wavelength (405 nm) was also avoided because of potential interference from hemolyzed samples (hemoglobin strongly absorbs at 410 nm—referred to as the “Soret” band).

ANALYTICAL RECOVERY AND PARALLELISM
Mean percentage recoveries (± 1SD) for samples supplemented with 90 and 50 mg/L purified prealbumin were 104.1% ± 4.3% and 100.1% ± 2.3%, respectively; the complete data are shown in Table 1. The mean recoveries (± 1SD) of endogenous prealbumin for each of the fixed proportions were 100% (± 3.1%), 101% (± 2.3%), 101% (± 2.1%), and 102% (± 2.1%). The results obtained for the parallelism study gave a linear regression of [Observed] = –1.5 + 1.02[Expected], with a coefficient of determination of 0.999.

IMPRECISION
There were no CV values significantly higher than the 5% determined by the $\chi^2$ analysis for either within-assay or total reproducibility in the four control serum pools (Table 2). The mean CV values (and ranges) obtained from duplicate analyses from the method comparisons were 1.59% (0–8.2%) for the Dimension analyzer assay and 1.22% (0–8.6%) for the Beckman Array assay.

STABILITIES OF CALIBRATION AND ON-BOARD REAGENTS
Table 2 also provides the estimates of total and within-assay reproducibility for both assessments of stability in each of the four control pools; CVs were 1–5%, except for a CV of 7.6% for the total reproducibility in the low pool for calibration stability. The daily pool means of the calibration stability (Fig. 3) showed no discernible trends over the duration of the study.

METHOD COMPARISON
A scattergram and a Passing and Bablok regression for the method comparison are shown in Fig. 4. The Dimension analyzer assay showed close agreement of both slope (1.00 ± 0.06) and intercept (7.0 ± 13) with the target values of 1.00 and zero within the ±95% confidence.
limits. An additional analysis (not shown), by the Bland–Altman method, demonstrated a random scatter in the relationship between method differences and averages, thus confirming the absence of bias.

**Interference**

Samples with added hemoglobin, bilirubin, and intralipid showed no significant change of results from those of the same samples in the absence of interferents (Table 3), with measured values all within 100% ± 5%. There also were no significant differences (P > 0.5 by Mann–Whitney) between the various sample collection conditions compared with serum obtained in plain tubes. At a concentration of 10 g/L albumin, the prealbumin assay showed a signal equivalent to the zero calibrator, thus demonstrating the negligible cross-reaction of albumin (data not shown).

**Assay Working Range and Sensitivity**

Using the CV cutoff of 10%, the precision profile of the form y = 6.37 – 4.3 \( \times 10^{-2} \) + 8.4 \( \times 10^{-5} \) (Fig. 5) gave an assay range which covered the range of calibration (0–550 mg/L). The detection limit (sensitivity), given by the intercept of the SD precision profile curve fit, of (the form \( y = 7.92 - 7.0 \times 10^{-2} + 1.9 \times 10^{-4} \)), was 8 mg/L prealbumin. The assay working range was taken as the values lying between the sensitivity and the uppermost standard, or 8–550 mg/L.

**Sample and Calibrator Stability**

There were no discernible changes in the sample pools or calibrators during the freeze-thaw cycles or during days 1 to 10 of incubations at 4°C and –20°C; recovery results all lay within ± 5% of the initial readings (data not shown).
The incubations at room temperature began to show appreciable variation of ± 20% from the running mean after 3 days in one of the samples and calibrators (Fig. 6). The incubations at room temperature began to show appreciable variation of ± 20% from the running mean after 3 days in one of the samples and calibrators (Fig. 6).

The incubations at room temperature began to show appreciable variation of ± 20% from the running mean after 3 days in one of the samples and calibrators (Fig. 6).

**Coupling Reproducibility**
The variation (CV) in signal over the three syntheses, at each concentration of prealbumin standard, was <5%.

### Table 3. Interferences from additions of hemoglobin, bilirubin, and triglyceride to six serum pools, expressed as mean recoveries and SD of measured results in the absence of interferent.

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Mean recovery, %</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7700</td>
<td>96</td>
<td>2.4</td>
</tr>
<tr>
<td>3850</td>
<td>97</td>
<td>3.6</td>
</tr>
<tr>
<td>1930</td>
<td>100</td>
<td>1.4</td>
</tr>
<tr>
<td>1000</td>
<td>98</td>
<td>2.4</td>
</tr>
<tr>
<td>500</td>
<td>99</td>
<td>1.1</td>
</tr>
<tr>
<td>Bilirubin, μmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>550</td>
<td>103</td>
<td>2.0</td>
</tr>
<tr>
<td>200</td>
<td>100</td>
<td>1.3</td>
</tr>
<tr>
<td>20</td>
<td>101</td>
<td>3.3</td>
</tr>
<tr>
<td>8.8</td>
<td>99</td>
<td>1.1</td>
</tr>
<tr>
<td>4.4</td>
<td>99</td>
<td>1.5</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.2</td>
<td>95</td>
<td>1.4</td>
</tr>
<tr>
<td>6.1</td>
<td>98</td>
<td>1.1</td>
</tr>
<tr>
<td>3.1</td>
<td>97</td>
<td>2.6</td>
</tr>
<tr>
<td>1.5</td>
<td>100</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Concentrations of prealbumin in the six pools were 80, 103, 118, 140, 180, and 250 mg/L.*
other analyzers, which, like the Dimension analyzer, may permit the use of in-house reagents.

The covalent coupling of proteins such as IgG has been shown in other established immunoassays to promote reagent stability (31, 36). This was reflected by the behavior of on-board reagent stability and calibration stability, where acceptable performance was observed for up to 40 days. The imprecision of the assay (CV, 1–5%) was similar to other clinical methods and direct immunoassays, such as PETIAs, that generally are more precise compared with their immuno-inhibition counterparts (35). Nutritional studies and/or reviews, composed of individual management of patients within defined groups (1–19), all indicate that this level of total precision (CV, <5%) is more than sufficient to be of diagnostic use, particularly in the critical area of interest (~40–170 mg/L) as well as including the higher reference range concentrations of 170–500 mg/L. The upper values of all reported reference ranges have been shown not to exceed 500 mg/L. Latex enhancement of immunoagglutination was observed to be an absolute requirement, because very poor signals were seen when free antibody was used, even in the presence of high PEG concentrations.

When the sample blank approach was used, the assay was unaffected by any of the added interferents: hemoglobin, bilirubin, or triglyceride (in the form of a lipid emulsion) at concentrations that might be expected in a routine clinical laboratory setting. Corresponding experiments that used baseline readings before sample addition demonstrated unacceptable photometric interferences, despite the relatively small proportion of sample (2 µL) present in a total reaction volume of 500 µL. In addition, the assay was observed to be valid for both serum and plasma collected by the methods most commonly used in any clinical practice. Close agreement of the Dimension analyzer PETIA with the reference method was demonstrated using calibrator values assigned by the Beckman method. Sample and calibrator stability indicated that specimen storage at room temperature for >3 days should be avoided. The small sample volume requirement of 2 µL (in a dead volume of 30 µL), the 10 mg/L detection limit, and the lack of significant interference from bilirubin or lipid makes the method to be suitable for pediatric use. In keeping with the performance of the Dimension AR or ES analyzers for other analytes, a throughput rate of 200 results/h may be achieved for this prealbumin assay. Quoted insert sheet details for commercially available methods, e.g., Beckman Array and Behring Nephelometer Analyzer® obtained from the manufacturers at the time of this study, indicate sample volumes and lower reportable limits of 150 µL and 70 mg/L for the Beckman Array and 50 µL and 20 mg/L for the Behring Nephelometer Analyzer.

In conclusion, an accurate, precise, reliable method for prealbumin has been developed and automated for use on the Dimension Clinical Chemistry Analyzer. The recommended format is defined in the open channels software, available for implementation onto the main instrument panel of diagnostic tests. Measurements of prealbumin and C-reactive protein can thus be integrated into a routine nutritional profile where the results for both may be obtained within 7 min. The method covers the entire concentration range of clinical interest with no predilution of sample.

P.H., D.J.N., H.T., Y.O., and C.L.D. were funded by a grant from Dade International, Dudingen, Switzerland. We thank SCIPAC, Sittingbourne, Kent, UK, for their generous gifts of purified prealbumin.

References