Clinical analysis of human serum proteins using the Beckman automated Paragon 2000 CZE system

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SUMMARY

Electrophoretic separation and quantitation of serum proteins, including para-
proteins, remain essential for the diagnosis and management of a variety of disorders.
We examined the Beckman automated Paragon 2000 capillary zone electrophoresis
(CZE) system and compared its electropherograms to those obtained by cellulose
acetate electrophoresis (CAE). Within-run CVs using seven different capillaries and
between-day CVs for CZE were <2% for albumin; they were <6% for α1-globulin,
α2-globulin, β-globulin, and γ-globulin. From 2.7 to 15.0 g/dl of total protein concentra-
tions, the same electrophoretic patterns could be observed. Comparisons of the five
principal bands obtained by CZE and cellulose acetate electrophoresis (CAE) gave
correlation coefficients from 0.8 to 0.9. On a quantitative basis in 55 specimens, albumin
by CZE was on average 10% lower than CAE, and α1-globulin and β-globulin were 93%
and 15% higher than by CAE, respectively (p<0.001). Based on our use of the Beckman
Paragon software and our reference values (percent of total protein) for CZE of 56.3 to
68.9% for albumin, 3.9 to 6.3% for α1-globulin, 4.8 to 8.4% for α2-globulin, 7.9 to 11.9%
for β-globulin, and 9.9 to 21.5% for γ-globulin, we found 75% to 100% agreement with
CZE; i.e., both methods gave either normal or abnormal results of the time. Patients
showing a gammopathy gave values that were higher by CZE 60% of the time.
Furthermore, the CZE system can resolve prealbumin, α1-acid glycoprotein, α1-
antitrypsin, hemopexin, transferrin, and complement besides to the usual five bands.

Key words: serum protein fraction, capillary electrophoresis, cellulose acetate electrophoresis, para-
proteins.

INTRODUCTION

More than 100 serum proteins have been identified to date by various separation techniques. One of the
simplest techniques for serum protein fraction is electrophoresis on a solid support such as cellulose acetate.
CAE electrophoresis is currently the most commonly used procedure in clinical laboratories for routine work,
though it has the disadvantage that proteins must soak into the cellulose acetate or other solid support. Follow-
ing electrophoresis, the bands must be visualized with a stain such as Ponceau S or amido black. The presence
of endosmosis is another disadvantage of CAE, and the commonly used stains give more color per gram of
albumin than per gram of an immunoglobulin. CZE, a recently developed method, minimizes these problems.
An automated system is available that carries out the sampling, electrophoretic separation, and quantitation
of the separated proteins, and that is a viable alternative to high-resolution agarose gel electrophoresis for
the determination of protein electrophoresis in a routine clinical laboratory. We examined the newly intro-
duced Beckman Instruments Paragon 2000 automated CZE analysis system, since this instrument was set to
separate the serum into five principle regions, even though the device permits the separation of the serum proteins into at least nine fractions: prealbumin, albumin, α-acid glycoprotein, α-antitrypsin, α2-globulin, hemopexin, transferrin, complement, and γ-globulin.

MATERIALS AND METHODS

Specimens
Blood was collected without an anticoagulant from 55 patients with various pathologies. We also collected serum specimens from 63 laboratory workers (31 males and 32 females, aged from 21 to 60 years old) who showed no chemical or hematological abnormalities. We used the 55 patients who were suspected of protein abnormalities in a comparison study of Paragon CZE and CAE, and the 63 normal individuals were used to estimate the reference range for the protein fractions by Paragon CZE. Human prealbumin, α1-acid glycoprotein, α1-antitrypsin, and transferrin (holo) were obtained from Sigma Chemical Co., St. Louis, MO, USA; human serum albumin was from Baxter Diagnostics Inc. (Dade Human Albumin Standard), Deerfield, IL, USA.

Capillary electrophoresis
Capillary electrophoresis was performed on a Beckman Instruments (Brea, CA) Paragon CZE 2000 system. Serum specimens were placed on the sample wheel of the instrument. The capillary tubing is composed of fused silica, having a 20 μm inner diameter and 20 cm length. The 150 mmol/l borate electrophoresis buffer (pH10.0) was purchased from Beckman. After the capillary was filled with buffer, about 60 nl of a dilution of 1 part serum and 20 parts buffer (with Beckman Signal Reagent) was injected into it and the electrophoresis was performed at an applied 9,000 V with the system thermostatted at 24°C. The addition of Signal Reagent was used for positioning marker peaks. Instrument settings for serum protein electrophoresis were as follows: 1 min conditioning time, 1 sec injection time, 4.3 min separation time, 0.5 min wash time, 0.5 min rinse time, 9,000 V, and 24°C. The applied potential produced the electrophoretic separation and also generated an electro-osmotic flow of the γ-globulins. A UV detector set at 214 nm was used; the absorbances are proportional to the concentration of peptide bonds. The absorbances monitored at 214 nm are neither affected by the presence of tyrosine, tryptophan, uric acid, or bilirubin, nor is there a difference between serum proteins.

The Paragon 2000 automatically selects pattern fractions by means of involved software package (Version 1.16). The instrument attempts to find a valley to delimit fractions, and the delimit placement is based on the percentage position between two marker peaks (Fig. 1; first and last markers). The software uses the passed valley information to place delimits in the first valley (reading from γ-globulin to albumin) in the expected location range. Furthermore, the Paragon CZE 2000 system can be coupled with an immunosubtraction blanking method to measure specific protein fractions, which will be reported elsewhere. Potential application of CZE to clinical samples, such as urine and cerebrospinal fluid, without sample pre-concentration has been demonstrated by Chen et al., whereas it needs to be further studied if these specimens are analyzed in Paragon CZE.

Cellulose acetate procedure (CAE)
CAE was performed on the Olympus AES 630 instrument (Olympus Co., Ltd., Tokyo, Japan) according to the manufacturer's procedure and reagents. The method is based on the standard electrophoresis procedure of the Japanese Electrophoresis Society. Total protein concentrations were assayed by using the biuret reaction with the reagents from Dia-Iatron Co., Ltd., and the instrument and procedure prescribed for the Hitachi 7250 automated analyzer.

RESULTS AND DISCUSSION

1. Serum protein electrophoresis by Paragon CZE
Electropherogram
Figure 1 shows a typical electropherogram of serum from a normal adult. Although at least nine fractions may be observed, the Paragon software (Version 1.16) separated them into five protein zones: albumin, α1-globulin, α2-globulin, β-globulin, and γ-globulin. Since it was proposed by this manufacturer that the Paragon CZE can identify prealbumin, α-acid glycoprotein, α-antitrypsin, hemopexin, transferrin, and complement besides the traditional five fractions, we confirmed their
migration times by adding various pure proteins. As described by others, fraction number 1 might be corresponded to prealbumin, 3 is α1-acid glycoprotein, 4 is α1-antitrypsin, 6 is hemopexin, 7 is transferrin, and 8 is complement. We certified the fractions of prealbumin, α1-acid glycoprotein, α1-antitrypsin, and transferrin by using pure proteins purchased from Sigma Chemical Co. Fraction number 8 was suspected to be complements by comparing the electropherograms before and after serum specimens are stored at room temperature for 24h (or at 4°C for three days), since this fraction disappeared after these treatments. Complements were very labile at room temperature. Because we could not obtain any pure hemopexin, peak 6 was not identified.

Reproducibility and linearity

The within-run and between-day reproducibilities are shown in Table 1. The CVs were acceptably small with the Paragon CZE procedure in comparison with that reported for CAE. We measured the migration time of each fraction from first and last marker peaks, which were found at 1.0 and 3.0 min, respectively. No distinct peaks were observed with Paragon CZE on prealbumin, α1-acid glycoprotein, or hemopexin. However, a duplicate analysis revealed that the migration times changed no more than 0.1 min.

We concentrated pooled serum up to 15.0 g/dl total protein levels by using Lyphogel (polyacrylamide gel, Gelman Sciences, Inc., Michigan, USA), and the concentrated serum was diluted to estimate the limit of detection with Paragon CZE. The same electropherograms were observed from 2.7 to 15.0 g/dl of total protein (Table 2).

Interference tests

Hemoglobin interferes in the determination of transferrin because it produces a peak at the same position as transferrin (see peak 7, Fig. 1). In a patient with an IgA monoclonal paraproteinemia, we found the abnormal fraction at positions 7 and 8, i.e., where transferrin and complement are found. Unfortunately, the electrophoretic patterns of serum and plasma were indistinguishable (electropherogram not shown), because fibrinogen could not be detected by Paragon CZE. Although Kurioka et al. reported that the migration time of each protein was based on pI value and molecular weight (MW), the fibrinogen (pI 5.8, MW 341 kDa) could not be distinguished from transferrin (pI 5.9, MW 90 kDa) in Paragon CZE with borate buffer. Lipidemic serum did not change the electropherogram up to 1,500 mg/dl triglyceride. Further studies are needed to identify these two fractions by Paragon CZE.

2. Comparison of serum protein electrophoresis by Paragon CZE and CAE

Correlation study

We compared the results of pathological sera measured by Paragon CZE with those by CAE and found good correlations: CZE = 1.055 (CAE) - 9.17, r = 0.956, Sy.x = 2.0 for albumin; CZE = 1.819 (CAE) + 0.32, r = 0.899, Sy.x = 1.0 for α1-globulin; CZE = 1.233 (CAE) - 1.58, r = 0.922, Sy.x = 1.3 for α2-globulin; CZE = 0.982 (CAE) + 1.58, r = 0.803, Sy.x = 1.0 for β-globulin; and CZE = 1.034 (CAE) + 0.47, r = 0.985, Sy.x = 0.8 for γ-globulin. The CZE results for albumin were on average 10% lower than the CAE (p < 0.001, Student’s paired t-test). The results for α1-globulin and β-globulin by the CZE method gave values that were on average 93% and 15% higher than the CAE procedure (p < 0.001), respectively. The above observation is consistent with that reported by Kurosu et al. They analyzed serum proteins by CZE with 50 μm x 50 cm untreated fused silica and CAE; the CZE results for albumin were on average 10% lower than the CAE (p < 0.001, Student’s paired t-test). The results for α1-globulin and β-globulin by the CZE method gave values that were on average 93% and 15% higher than the CAE procedure (p < 0.001), respectively. The above observation is consistent with that reported by Kurosu et al. They analyzed serum proteins by CZE with 50 μm x 50 cm untreated fused silica and CAE; the CZE results for albumin were also on average 7% lower than CAE and for α1-globulin on average 53% higher than CAE. Although the reasons for the higher α1-globulin results are not known, others have reported that the high sialic acid content of α1-acid glycoprotein interfered with the binding of dyes used to quantitate the protein fraction by CAE.

Table 1. Reproducibility of Paragon 2000 capillary zone electrophoresis.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Within-run reproducibility</th>
<th>Between-day reproducibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 capillaries</td>
<td>10 days</td>
</tr>
<tr>
<td></td>
<td>mean±SD (%)</td>
<td>mean±SD (%)</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>CV (%)</td>
</tr>
<tr>
<td>albumin</td>
<td>58.9±0.6</td>
<td>59.1±1.0</td>
</tr>
<tr>
<td>α1-globulin</td>
<td>5.3±0.1</td>
<td>5.3±0.3</td>
</tr>
<tr>
<td>α2-globulin</td>
<td>9.0±0.1</td>
<td>7.2±0.2</td>
</tr>
<tr>
<td>β-globulin</td>
<td>11.1±0.2</td>
<td>13.3±0.2</td>
</tr>
<tr>
<td>γ-globulin</td>
<td>15.7±0.4</td>
<td>15.1±0.6</td>
</tr>
</tbody>
</table>

All aliquots of pooled serum were stored at −20°C.

Table 2. Effect of total protein concentration on Paragon 2000 capillary zone electrophoresis.

<table>
<thead>
<tr>
<th>Five ordinary fractions</th>
<th>Total protein (g/dl)</th>
<th>15.0</th>
<th>10.8</th>
<th>5.4</th>
<th>2.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>albumin</td>
<td>49.6</td>
<td>49.2</td>
<td>49.1</td>
<td>48.9</td>
<td></td>
</tr>
<tr>
<td>α1-globulin</td>
<td>6.2</td>
<td>7.4</td>
<td>7.2</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>α2-globulin</td>
<td>10.6</td>
<td>9.7</td>
<td>9.5</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>β-globulin</td>
<td>11.6</td>
<td>11.4</td>
<td>11.3</td>
<td>11.7</td>
<td></td>
</tr>
<tr>
<td>γ-globulin</td>
<td>22.0</td>
<td>22.3</td>
<td>22.8</td>
<td>23.1</td>
<td></td>
</tr>
<tr>
<td>minor fractions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prealbumin</td>
<td>0.6</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>α1-acid glycoprotein</td>
<td>2.7</td>
<td>2.7</td>
<td>2.8</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>α1-antitrypsin</td>
<td>4.8</td>
<td>4.7</td>
<td>4.4</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>hemopexin</td>
<td>1.9</td>
<td>1.8</td>
<td>1.7</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>transferrin</td>
<td>5.1</td>
<td>5.1</td>
<td>5.3</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>complement</td>
<td>4.6</td>
<td>4.5</td>
<td>4.3</td>
<td>4.8</td>
<td></td>
</tr>
</tbody>
</table>

Concentrated serum was diluted and the electropherogram obtained. Values indicate a percentage of each fraction.
Reference range
To estimate a reference range because α1-globulin was markedly higher in the Paragon CZE, we used sera from the 63 normal adults. Gaussian distributions of the values were observed for the standard five protein zones. Using the mean and SD (mean±SD in parentheses), we estimated the reference ranges for Paragon CZE to be 56.3 to 68.9% for albumin (62.6±3.1), 3.9 to 6.3% for α1-globulin (5.1±0.6), 4.8 to 8.4% for α2-globulin (6.6±0.9), 7.9 to 11.9% for β-globulin (9.9±1.0) and, 9.9 to 21.5% for γ-globulin (15.7±2.9). These reference intervals by Paragon CZE were 10% lower for albumin, 93% higher for α1-globulin, and 15% higher for α2-globulin than those for CAE; however, the ranges for α2-globulin and γ-globulin did not differ from the values by CAE. Our mean±2SD ranges for the five protein zones were much like those proposed by Ueno et al.15)

Because the CZE system software automatically segregates the electropherogram into only five zones, although more than five protein fractions are present, we decided to estimate the protein concentrations of all available fractions, i.e., prealbumin, α1-acid glycoprotein, α1-antitrypsin, hemopexin, transferrin, and complement. Here their concentrations were calculated from a relative percent of each fraction and total protein concentration. Serum proteins have nearly the same reactivities with biuret reagent. In 14 normal sera obtained from laboratory workers in good health, mean (SD, percent mean, percent SD) concentrations were prealbumin 33mg/dl (9mg/dl, 0.5%, 0.1%); α1-acid glycoprotein 161mg/dl (23mg/dl 2.0%, 0.3%); α1-antitrypsin 231mg/dl (45mg/dl, 2.8%, 0.3%); hemopexin 101mg/dl (10mg/dl, 1.5%, 0.4%); transferrin 358mg/dl (92mg/dl, 4.6%, 0.9%); and complement 312mg/dl (114mg/dl, 3.8%, 0.8%). These results are nearly the same as data obtained previously by nephelometric analysis.10 The minor fractions could be reported besides the five main zones.17) Yashiro18) and Oikawa19) also reported good correlations between CZE and nephelometric analysis of the complement (r=0.524, p<0.001) and the transferrin (r=0.810, p<0.001). We can obtain analytical recovery of four minor fractions by adding pure proteins to the serum pool (Table 3). The mean analytical recovery was 100% for prealbumin, 84% for α1-acid glycoprotein, 54% for α1-antitrypsin, and 83% for transferrin.

Patient study
Using CAE and Paragon CZE, we compared sera from 55 patients and employed the method-specific references ranges. Agreement was examined from the number of abnormal results in each protein zone. Of 55 patients, 49 showed at least one protein abnormality by either method. We found good agreement in cases of hypoalbuminemia, hyper-α2-globulinemia and, hyper-γ-globulinemia. The agreement was poor in cases of hypo-γ-globulinemia, hyper-α1-globulinemia, hypo-β-globulinemia, and hyper-β-globulinemia (Table 4). When we also compared the electrophoresis results on the same patients with the Paragon software (out of 10% greater or 10% lower from the mean±2SD reference range), a better agreement was observed for the hyper-α1-globulinemia and hyper-β-globulinemia. We

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Prealbumin (mg/dl)</th>
<th>α1-acid glycoprotein (mg/dl)</th>
<th>α1-antitrypsin (mg/dl)</th>
<th>Transferrin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Added</td>
<td>Measured</td>
<td>Initial</td>
</tr>
<tr>
<td>1</td>
<td>60</td>
<td>111</td>
<td>80</td>
<td>94</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>55</td>
<td>60</td>
<td>104</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>28</td>
<td>45</td>
<td>102</td>
</tr>
<tr>
<td>Mean recovery</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Parentheses indicate analytical recovery obtained by adding 0.15 ml of a pure protein solution to 0.15 ml of serum pool, and detecting four bands.

Table 4. Agreement of electropherograms of proteins in serum from 55 patients by Paragon 2000 capillary zone electrophoresis (CZE) and cellulose acetate electrophoresis (CAE).

<table>
<thead>
<tr>
<th>Protein</th>
<th>Number of sera beyond the method-specific mean±2 SD reference interval</th>
<th>Number of sera beyond±10% of the mean±2 SD reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower than CAE Agreement</td>
<td>Greater than CAE Agreement</td>
</tr>
<tr>
<td>albumin</td>
<td>17</td>
<td>14 (82)</td>
</tr>
<tr>
<td>α1-globulin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>α2-globulin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>β-globulin</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>γ-globulin</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

*Agreement* means abnormal results in each protein zone by the two methods.
*Parentheses indicate percentage of number of sera agreeing with the CAE results.
recommend the use of Paragon software in besides the mean±2SD reference ranges for electrophoresis results. However, poor coincidence in hypo-γ-globulinemia remained (60% agreement), and this also needs further investigation.

Furthermore, reliable reference values for Paragon CZE for the individual proteins would facilitate the diagnoses in cases of hypoproteinemia (lowered prealbumin), hypoalbuminemia, an acute phase pattern with inflammation (elevated α1-acid glycoprotein, α2-antitrypsin, and complement), nephrotic syndrome, iron deficiency anemia, hemolytic diseases (lowered hemopexin and transferrin), autoimmune diseases (lowered complement), hypogammaglobulinemia, or a gammopathy, myeloma, or paraproteinemias, and increased polyclonal immunoglobulins because of chronic active hepatitis.

We concluded that Paragon CZE is a useful tool; it is fast, efficient, and easy to employ and it provides more information than the classical five-band CAE. It is not affected by the dye binding efficiency of the proteins; thus more accurate protein concentrations can be obtained.

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REFERENCES