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Automated Serum Protein Electrophoresis by Capillarys®

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Capillary zone electrophoresis (CZE) of serum proteins is increasingly gaining impact in clinical laboratories. In this report, we evaluate automated capillary zone electrophoresis by Capillarys® (Sebia, France). Within-run and between-run imprecision for the five electrophoretic fractions was <2% and <6%, respectively. Data obtained with Capillarys correlated with results obtained with agarose gel electrophoresis and Paragon CZE® 2000 (Beckman Coulter, USA). Analysis of serum obtained from patients with inflammation, nephrotic syndrome, bisalbuminemia, and α_1-antitrypsin deficiency revealed that Capillarys was able to detect these abnormalities. Two hundred thirty eight samples were analyzed by agarose gel electrophoresis, Capillarys, capillary electrophoresis using Paragon CZE 2000 system, and immunofixation. Sample selection was based on the presence of a disturbed morphology (*e.g.*, spike) of the protein profile or hypogammaglobulinemia on agarose gel electrophoresis and/or Capillarys. Immunofixation revealed the presence of a monoclonal protein, oligoclonal bands, polyclonal pattern, and a normal profile in, respectively, 89, 66, 19, and 64 samples. With Capillarys, Paragon, and agarose gel electrophoresis, a spike and/or disturbed morphology of the profile was found in 222, 182, and 180 samples, respectively. In these samples, immunofixation was negative in 73 (33%), 46 (25%), and 39 (22%) samples, respectively. These data indicate that Capillarys has a lower specificity than agarose gel electrophoresis and Paragon 2000. Of the 89 samples with a monoclonal protein, Capillarys, Paragon, and agarose gel electrophoresis failed to detect, respectively, three, three, and one monoclonal protein(s). Interferences by radio-opaque agents, complement degradation products, fibrinogen, and triglycerides are described. In conclusion, automated capillary zone electrophoresis with Capillarys provides for reproducible, rapid, and reliable serum electrophoresis. Clin Chem Lab Med 2003; 41(5):704–710

Key words: Serum proteins; Monoclonal proteins; Electrophoresis; Capillary zone electrophoresis.

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Abbreviations: AGE, agarose gel electrophoresis; CZE, capillary zone electrophoresis.

Introduction

Serum protein electrophoresis is an established and effective disease screening tool in clinical chemistry. The technique is widely used, especially for the detection of monoclonal (M)-proteins. Traditional methods use agarose gels or cellulose acetate membranes as the separation bed. More recently, capillary zone electrophoresis (CZE) of serum proteins has been increasingly used in clinical laboratories (1–11). In the late 1990's, the Beckman Coulter Paragon CZE® 2000 Clinical Capillary Electrophoresis instrument (Beckman-Coulter, Brea, CA, USA) was introduced. This fully automated multi-channel instrument that is dedicated to the separation of serum proteins is particularly helpful in clinical laboratories that have to deal with a large daily serum protein electrophoresis workload. Lately, a second multi-channel automated system, the Capillarys® (Sebia, Issy-les Moulinaux, France), has been launched. In this study, the Capillarys is evaluated and compared to agarose gel electrophoresis (AGE) and Paragon 2000 (Beckman-Coulter, Brea, CA).

Materials and Methods

CZE analysis

The Capillarys system (Sebia, Issy-les-Moulineaux, France) was operated according to the manufacturer's instructions under software version 1.4.1. The instrument has eight fused silica capillaries (17 cm in length and 25 μm ID). The buffer (borate and additives) is pH 10 and sample is diluted 1:10. Detection voltage is 9 kV. Separation is carried out at 35 °C and takes 2.5 min. Ultraviolet detection at 200 nm is used for direct quantification of the peptide bonds. When the samples are analyzed in batch, Capillarys has a throughput of 100 samples/h.

The Paragon 2000 instrument (Beckman-Coulter, Brea, CA) was operated according to manufacturer's instructions. The instrument has seven fused silica capillaries (20 cm in length and 25 μm ID). The buffer (borate) is pH 10 and sample is diluted 1:20. Detection voltage is 9 kV. Separation is carried out at 24 °C and takes 4.3 min. Ultraviolet detection at 214 nm is used for direct quantification of the peptide bonds. The software version used was 1.5 for all analyses except for the analyses shown in Figures 5, 6, and 7. When the samples are analyzed in batch, Paragon CZE 2000 has a throughput of 38 samples/h.

Agarose gel electrophoresis and immunofixation

Semi-automated AGE and immunofixation were performed with the Hydrasys automate (Sebia, Issy-les-Moulineaux, France), using Hydragel protein (five band) and Hydragel im-

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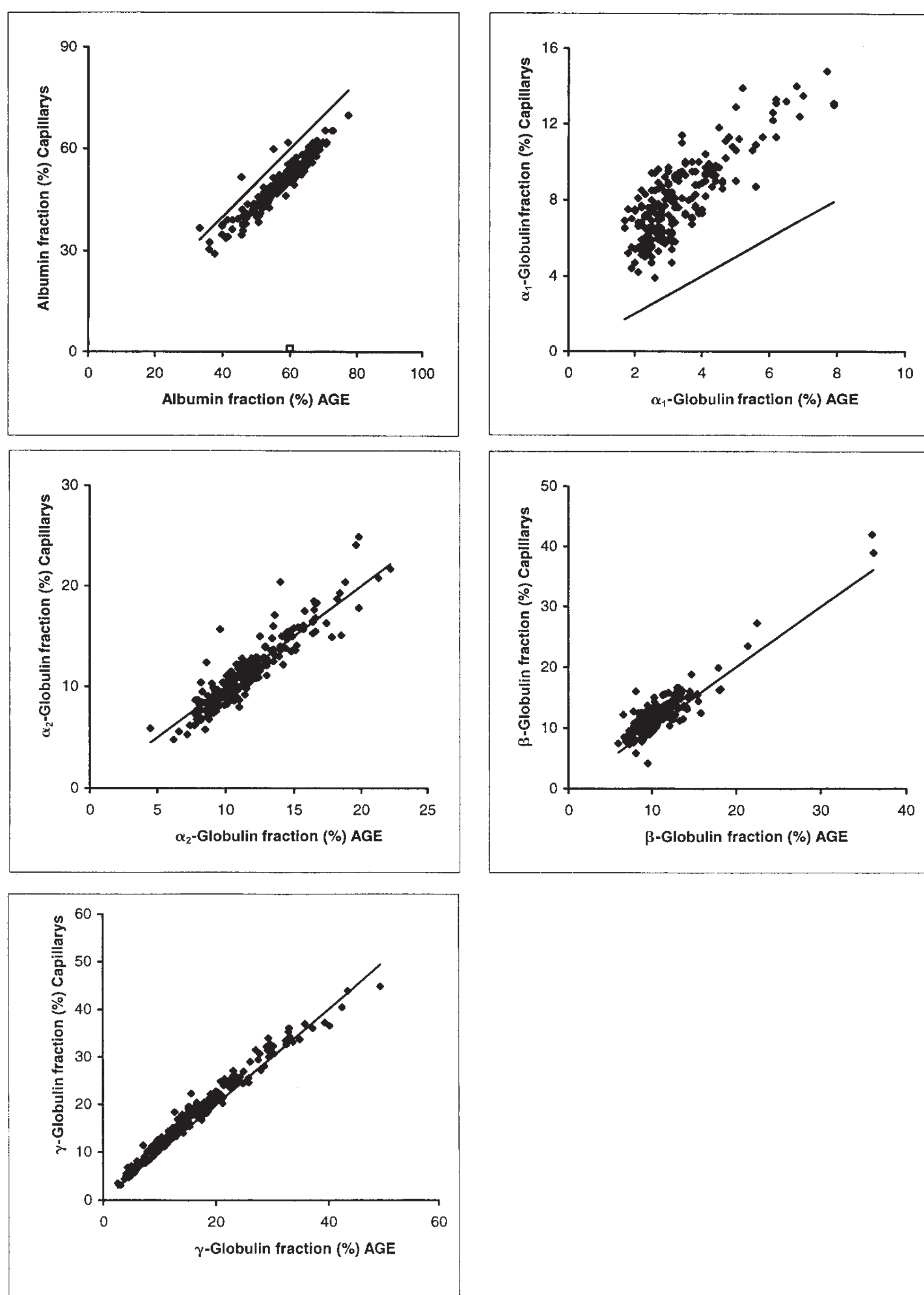

Figure 1 Correlation between AGE and CZE. Correlation was evaluated using 225 samples. The panels show the correlation between semi-automated AGE and CZE and Capillarys for the five electrophoretic protein fractions. The lines on the graphs represent the line of identity.

munofixation (Penta for screening and G, A, M, K, L for identification) gels (Sebia, Issy-les-Moulineaux, France).

Specimens

Specimens used for the studies were patient samples submitted to the laboratory for serum protein analysis. In addition, specimens from 50 healthy adults (31 males and 19 females) were obtained from blood donors (Red Cross, Belgium). When indicated, selected patient samples were used.

Statistical analysis

Statistical analysis was performed with Microsoft® Excel 2000, Analyse-it, version 1.62, 2002. Passing-Bablok analysis was used for calculating method comparison statistics and Pearson analysis for calculating correlation coefficients.

Results and Discussion

Reproducibility

Within-run imprecision studied using a sample from a healthy adult (n = 20) was <2% for all fractions with the Capillarys. Between-run imprecision was <6% (n = 10). The reproducibility is comparable to the reproducibility reported for Paragon CZE 2000 (2, 3).

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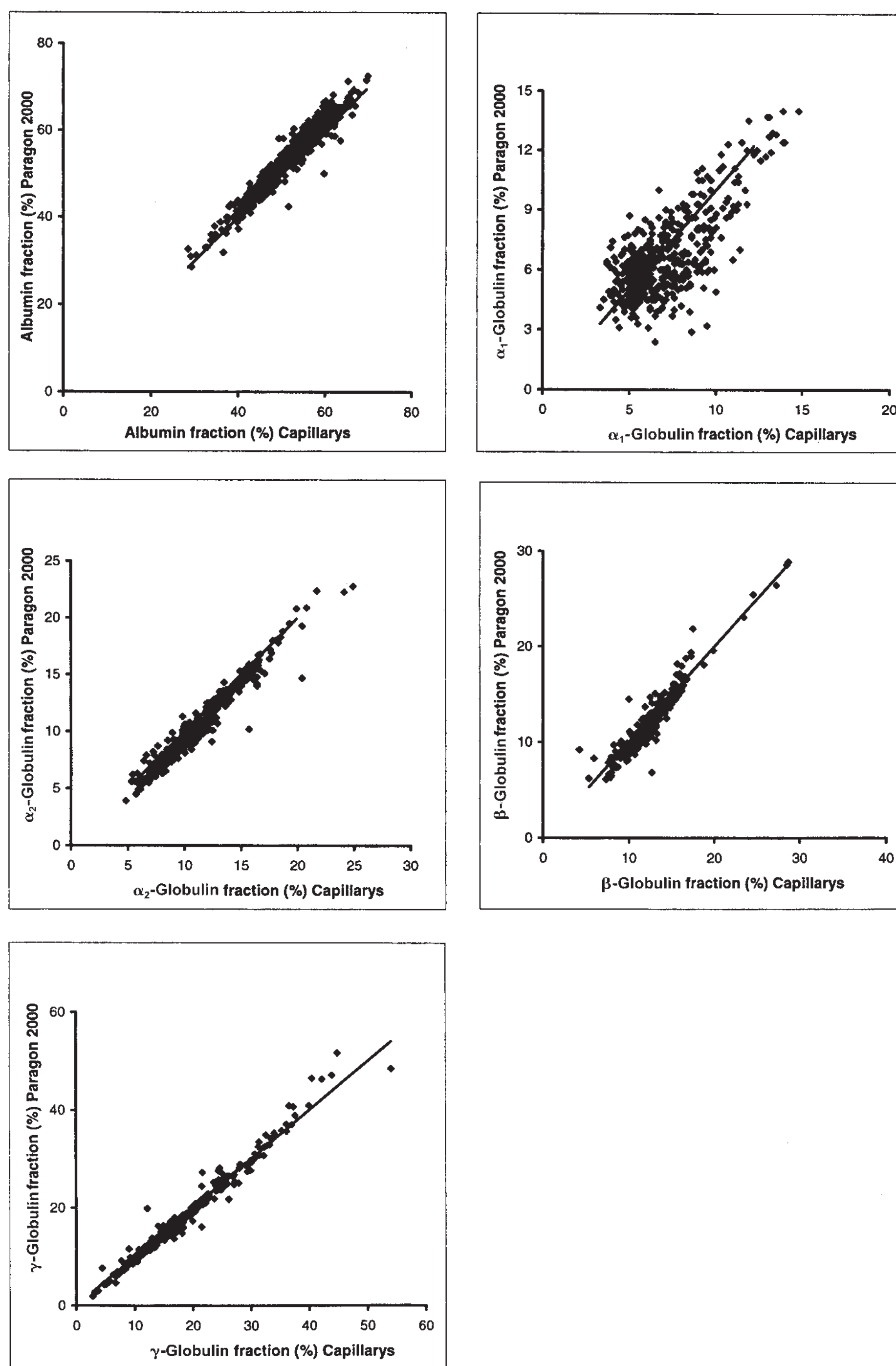

Figure 2 Correlation between Capillarys and Paragon 2000 systems. Correlation was evaluated using 434 samples. The panels show the correlation between semi-automated Capillarys and Paragon 2000 for the five electrophoretic protein fractions. The lines on the graphs represent the line of identity.

Method comparison study

To compare AGE with Capillarys, 225 serum samples were analyzed using Hydrasys and on Capillarys. The correlation is shown in Figure 1. The interval of relative percentage studied was 4.2, 1.2, 3.2, 6.0, and 4.3 times the reference interval (see below) for the albumin, α_1-globulin, α_2-globulin, β-globulin, and γ-globulin fraction, respectively. Passing-Bablok method comparison between AGE (X-values, reference) and Capillarys (n = 225) revealed a slope and intercept of, respectively, 0.976 (95% CI 0.942 to 1.008) and −6.083 (95% CI −7.974 to −4.038) for the albumin fraction, 2.167 (95% CI 2 to 2.421) and 0.667 (95% CI −0.086 to 1.1) for the α_1-globulin fraction, 1.179 (95% CI 1.121 to 1.239) and −2.386 (95% CI −3.028 to −1.706) for the α_2-globulin fraction, 1.073 (95% CI 1 to 1.167) and 0.722 (95% CI −0.3 to 1.5) for the β-globulin fraction, and 1.017 (95% CI 0.995 to 1.04) and 1.36 (95% CI 1.004 to 1.677) for the γ-globulin fraction. The r-values were 0.95, 0.83, 0.93, 0.91, and 0.99 for albumin, α_1-globulin, α_2-globulin, β-globulin, and γ-globulin fraction, respectively. Cusum test re-

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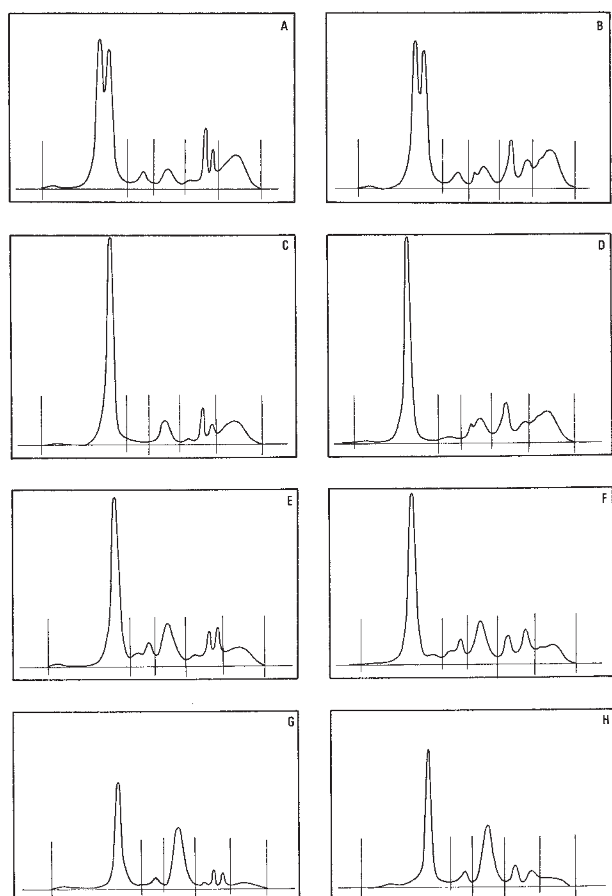

Figure 3 Comparison of electrophoretic patterns in various conditions. CZE electropherograms obtained with Paragon 2000 (Panels A, C, E, and G) and with Capillarys system (Panels B, D, F, and H) in patients with dysproteinemia; bisalbuminemia (Panels A and B), α_1-antitrypsin deficiency (Panels C and D), an acute reactive process (Panels E and F), and a nephrotic syndrome (Panels G and H).

vealed significant deviation from linearity for the α_1-globulin ($p < 0.01$) and the γ-globulin fraction ($p < 0.05$). A constant bias was detected for the albumin, α_2-globulin and γ-globulin fraction. A proportional bias was observed for the α_1-and α_2-globulin fraction. Marked differences found in the α_1-globulin fraction are related to the high sialic acid content of the α_1-acid glycoprotein, which interferes with the binding of dyes in gel-based systems. The UV absorption method used in CZE is not affected by these sugar moieties. Similar findings have been previously reported with the Paragon 2000 (2).

Four hundred thirty four samples were analyzed with Capillarys and with Paragon. The interval of relative percentage studied was several times the reference interval (see below), namely 3.9, 2.3, 3.6, 7.6, and 4.7 for the albumin, α_1-globulin, α_2-globulin, β-globulin, and γ-globulin fraction, respectively. Passing-Bablok method comparison between Capillarys (X-values, reference) and Paragon revealed a slope and intercept of, respectively, 1.036 (95% CI 1.011 to 1.062) and −0.296 (95% CI −1.608 to 0.988) for the albumin fraction, 0.944 (95% CI 0.871 to 1) and 0.053 (95% CI −0.4 to 0.581) for the α_1-globulin fraction, 1.023 (95% 1 to 1.043) and −0.987 (95% CI −1.188 to −0.7) for α_2-globulin fraction, 1.098 (95% CI 1.069 to 1.129) and −1.688 (95% CI −2.042 to −1.343) for β-globulin fraction, and 1.02 (95% CI 1.009 to 1.029) and −0.613 (95% CI −0.754 to −0.452) for the γ-globulin fraction. The r-values were 0.97, 0.73, 0.98, 0.97, and 0.99 for albumin, α_1-globulin, α_2-globulin, β-globulin, and γ-globulin fraction, respectively. The correlation is shown in Figure 2. Cusum test revealed significant deviation from linearity for the albumin ($p < 0.05$) and α_2-globulin ($p < 0.01$) fraction. The poor correlation that was found for the α_1-globulin fraction between Capillarys on the one hand, and Paragon 2000 and AGE on the other, is related to the fact that the delimitation between the albumin fraction and the α_1-globulin fraction might be difficult with Capillarys. A constant bias was detected for the α_2-, β- and γ-globulin fraction. A proportional bias was detected for the albumin, β- and γ-globulin fraction.

Analysis of samples obtained from 50 adult blood donors using the Capillarys system gave a 2.5th and 97.5th percentile of 55.6% and 66%, respectively, for the albumin fraction, 2% and 6.9% for the α_1-globulin fraction, 5.9% and 11.4% for the α_2-globulin fraction, 9.4% and 14.4% for the β-globulin fraction, and 8% and 18.8% for the γ-globulin fraction.

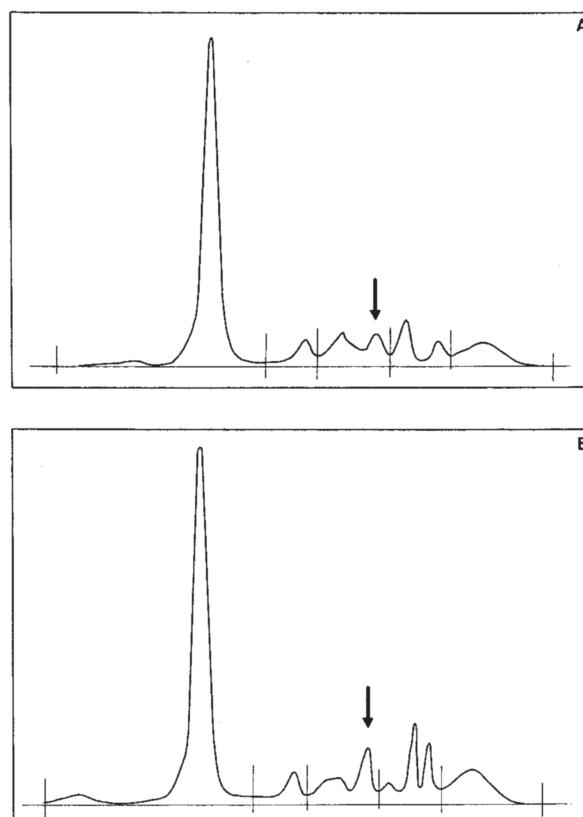

Figure 4 Capillarys (Panel A) and Paragon 2000 (Panel B) CZE electropherogram of a serum sample that contained a radio-opaque agent (Omnipaque®). The abnormal peak due to the radio-opaque agent is indicated by an arrow.

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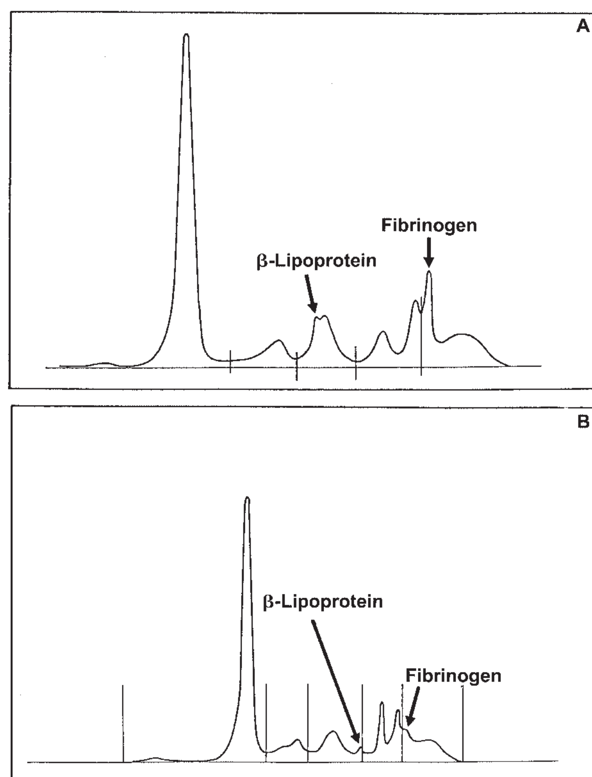

Figure 5 Capillarys (Panel A) and Paragon 2000 (software version 1.6) (Panel B) electropherogram of plasma samples. The samples that have been analyzed on the two instruments were not identical. Position of the β-lipoprotein and fibrinogen is indicated (manufacturer, personal communication).

Analysis of samples from dysproteinemic patients

Figure 3 shows the electropherograms obtained with Capillarys and with Paragon 2000 for selected serum samples with dysproteinemia (bisalbuminemia, α_1-antitrypsin deficiency, acute reactive process and nephrotic syndrome). Bisalbuminemia was easily detected by both instruments. Deficiency of α_1-antitrypsin resulted in the lack of the specific α_1-antitrypsin peak within the α_1-globulin fraction. It should be pointed out, however, that CZE shows no relationship between α_1-antitrypsin phenotype and the concentration of the α_1-globulin fraction (12). Neither Paragon nor Capillarys was able to distinguish α_2-macroglobulin from haptoglobin in the α_2-globulin fraction.

A small but distinctive restriction in the anodal portion of the γ-region was seen with the Capillarys system in Figure 3B, D, and F. These occasionally seen small bands are not caused by a monoclonal protein (negative immunofixation).

Detection of monoclonal proteins

Two hundred thirty eight samples were analyzed using AGE, Capillarys, Paragon 2000, and immunofixation. The samples were selected from sequential patient samples submitted to the laboratory for protein electrophoresis. The selection was based on the presence of a spike or a disturbed morphology of the protein profile (bands with restricted mobility) with AGE and/or Capillarys. Samples in which neither AGE nor Capil-

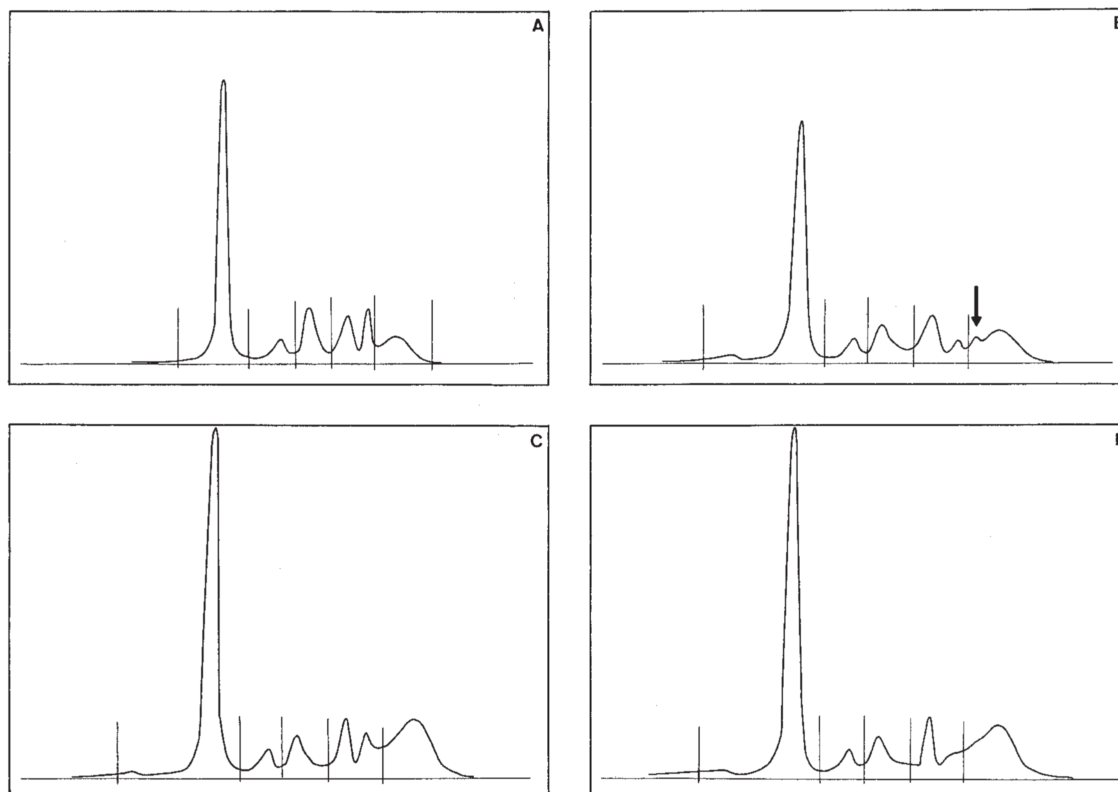

Figure 6 Effect of complement degradation on CZE analysis. A serum sample was analyzed on Capillarys (Panels A and B) or on Paragon 2000 CZE (software 1.6) system (Panels C and D) and on Capillarys system (Panels C and D) either immediately after collection of the sample (Panel A and C) or after an incubation of the sample at 37 °C for 5 days (Panels B and D). The arrow indicates the position of the complement degradation products.

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larys showed an abnormal spike but in which hypogammaglobulinemia was present were included as well. Immunofixation analysis revealed the presence of a monoclonal protein in 89 samples, oligoclonal bands in 66 samples, and a polyclonal profile in 19 samples. In 64 samples, no monoclonal protein was revealed by immunofixation analysis.

Capillarys, Paragon, and AGE revealed a disturbed morphology of the protein profile in, respectively, 73, 46, and 39 samples out of 83 samples, in which immunofixation showed no abnormalities or a polyclonal increase of the γ-globulin fraction. Of the 89 samples with a monoclonal protein, both Capillarys and Paragon 2000 (software version 1.5) failed to detect two low-concentration monoclonal proteins (IgGκ and IgMκ) that had been detected by AGE. Besides, Paragon missed one small monoclonal protein (migrating in the slow γ-region) that was revealed by Capillarys and by AGE. The failure of Paragon to detect such monoclonal proteins that migrate in the slow γ-region has been described previously (11). Capillarys missed one small β-migrating monoclonal protein that was revealed by Paragon.

Capillarys, Paragon, and AGE revealed disturbed morphology of the protein profile in, respectively, 63, 50, and 54 of the 66 samples in which immunofixation revealed oligoclonal bands.

With Capillarys, a spike and/or a disturbed morphology of the protein profile was found in 222 samples. Immunofixation analysis of these samples revealed that in 73 (33%) samples there was no evidence of the presence of oligoclonal bands or the presence of a monoclonal protein. With Paragon and with AGE, a spike and/or disturbed morphology of the protein profile was found in 182 and in 180 samples, respectively. In these samples, immunofixation was negative in 46 (25%) and 39 (22%) samples, respectively. These data indicate that for the detection of monoclonal proteins Capillarys has a lower specificity than AGE and Paragon 2000. Such lower specificity might cause needless increase in immunofixation studies.

Interferences

Detector interference

Radio-opaque agents. Interference by radio-opaque agents has been described previously (13–15) on the Paragon 2000 system. These agents interfere with CZE analysis in which ultraviolet detection at 200 nm (Capillarys) or 214 nm (Paragon 2000) is used for direct protein quantification of the peptide bonds. Figure 4 shows the Capillarys and Paragon CZE electropherogram of a sample that contains Omnipaque®, a radio-opaque agent. An abnormal peak due to the radio-opaque agent is seen in the α_2-globulin region. Such interference is not observed in conventional gel-based methods, where quantification of the protein fractions is based on dye binding.

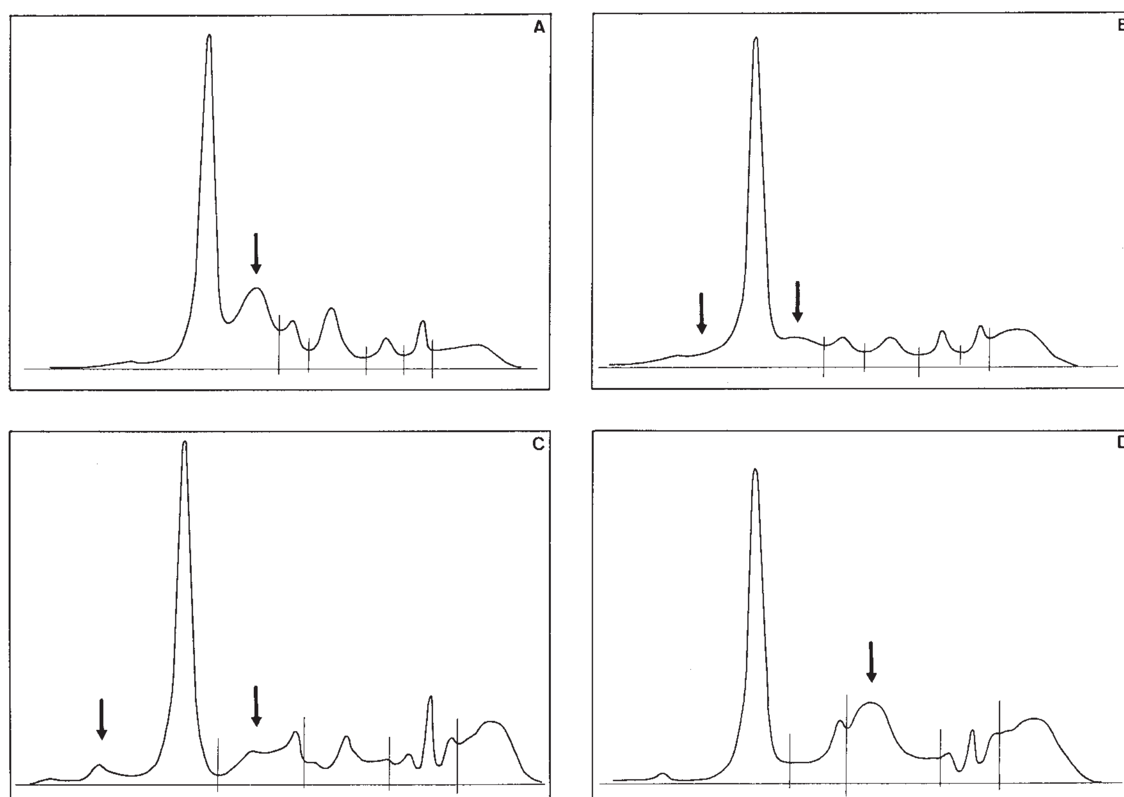

Figure 7 Effect of triglycerides on CZE analysis. Serum samples containing high triglyceride concentration were analyzed with Capillarys (Panel A and B) and with Paragon 2000 (software 1.6) system (Panel C and D). The triglyceride concentration was 4132 mg/dl (46.6 mmol/l; Panel A), 858 mg/dl (9.68 mmol/l; Panel C), and 2231 mg/dl (25.18 mmol/l; Panel B and D). The arrows indicate the interference.

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Sample type and pretreatment

Anticoagulant. Fibrinogen is revealed with the Capillarys system. With Paragon it is revealed with software version 1.6 but not with earlier software. The location of fibrinogen is illustrated in Figure 5. The fibrinogen band may interfere with the interpretation as it obscures an important part of the β-γ-region in an incompletely clotted serum or in heparinized samples.

In vitro hemolysis. *In vitro* hemolysis resulted in disturbed morphology of the α_2-globulin fraction and in an increased and disturbed peak at the transferrin position (data not shown).

Complement. The effect of complement degradation on CZE was studied by incubating a sample at 37 °C for 5 days. Such treatment results in a complete breakdown of C3 (16). CZE analysis was performed before and after incubation at 37 °C. The results are shown in Figure 6 and illustrate that complement degradation results in the loss of the C3 peak. With Capillarys, a small peak at the anodal site of the γ-globulin fraction appeared.

Lipid. β-Lipoprotein is revealed with Capillarys. It is also seen with Paragon 2000 with software version 1.6 but not in earlier operating conditions (*e.g.*, software version 1.5). The location of β-lipoprotein in the electrophoretic profile for each system is illustrated in Figure 5. Analysis of samples that contain elevated levels of triglycerides resulted in a disturbed morphology and increased relative percentage of the prealbumin region and/or the albumin region and/or the α_1-globulin region and/or the α_2-globulin region in both systems. Some examples of interferences by triglycerides on the Capillarys and Paragon are shown in Figure 7.

General conclusions

Automated CZE with Capillarys provides reproducible and rapid serum electrophoresis. It is particularly useful in clinical laboratories that have a relatively large daily workload. The Capillarys instrument is easy to operate and the software is user-friendly. CZE analysis by Capillarys is an alternative to traditional agarose gel-based zone electrophoresis and may lead to savings related to automation. However, the decreased specificity for the detection of monoclonal proteins may result in increased costs for immunofixation analysis.

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