Clinical Chemistry 49:11
1909–1915 (2003)

Automation and Analytical Techniques

Automated Multicapillary Electrophoresis for Analysis of Human Serum Proteins

Cécile Gay-Bellile,[1] Djaouida Bengoufa,[2] Pascal Houze,[1] Didier Le Carrer,[3] Mourad Benlakehal,[1] Bernard Bousquet,[1] Bernard Gourmel,[1] and Thierry Le Bricon[1*]

Background: **We evaluated a new, automated multicapillary zone electrophoresis (CE) instrument (Capillarys®, 4.51 software version; Sebia) for human serum protein analysis.**

Methods: **With the Capillarys β1-β2+® reagent set, proteins were separated at 7 kV for 4 min in 15.5 cm × 25 μm fused-silica capillaries (n = 8) at 35.5 °C in a pH 10 buffer with online detection at 200 nm. Serum samples with different electrophoretic patterns (n = 265) or potential interference (n = 69) were analyzed and compared with agarose gel electrophoresis (AGE; Hydrasys®-Hyrys®, Hydragel protein(e) 15/30® reagent set; Sebia).**

Results: **CVs were <3.5% for albumin, <11% for α_1-globulin, <4.1% for α_2-globulin, <7.4% for β-globulin, and <5.8% for γ-globulin (3 control levels); measured throughput was 60 samples/h. In patients without paraprotein (n = 116), the median differences between CE and AGE were −5.4 g/L for albumin, 4.0 g/L for α_1-globulin, 0.7 g/L for α_2-globulin, 0.6 g/L for β-globulin (P <0.001 for all fractions), and −0.1 g/L for γ-globulin (not significant). More samples had at least one γ-migrating peak detected by CE (n = 135 vs 130; paraprotein detection limit, ~0.5–0.7 g/L), but fewer were quantified (n = 84 vs 91) because of γ- to β-migration shifts. There was a 1.2 g/L median difference between CE and AGE for γ-migrating paraprotein quantification (n = 69; P <0.001). Several ultraviolet-absorbing substances (lipid emulsion, hemoglobin) or molecules (contrast agent, gelatin-based plasma substitute) induced CE artifacts.**

Conclusions: **The Capillarys instrument is a reliable CE system for serum protein analysis, combining advantages of full automation (ease of use, bar-code identification, computer-assisted correction of α_1-globulins) with high analytical performances and throughput.**

Analysis of serum proteins by electrophoresis is routinely used to screen and monitor not only B-cell malignancies *(1, 2)*, but also immune and, to a lesser extent, inflammatory responses. In clinical chemistry laboratories, standard electrophoresis is usually performed using the agarose gel as a migration support (AGE).[4] Despite ready-to-use commercial reagent sets and semiautomated electrophoresis systems, this technique remains labor-intensive, with resulting limited analytical performances and throughput.

During the last decade, capillary electrophoresis (CE) has emerged as a powerful analytical tool *(3, 4)*. High voltage applied in a thin (internal diameter <100 μm) fused-silica tube allows fast and efficient separation of ionized molecules or complexes *(5)*. Protein analysis is usually performed in free solution with use of highly basic buffers to minimize interactions with capillary walls. Direct quantification is obtained in the low ultraviolet (UV) range (~200 nm via the peptide bonds) at the cathodic end of the capillary *(6, 7)*. The first automated multicapillary instrument designed for routine serum protein analysis by CE, the Paragon CZE2000® (Beckman), was commercialized in 1994. Its advantages include high precision and resolution, slightly improved sensitivity for paraprotein detection (vs AGE), use of small amounts of

[1] Laboratoire de Biochimie A et de Neurobiologie and [2] Laboratoire d'Immunologie et d'Histocompatibilité, Hôpital St-Louis AP-HP, 1 avenue Claude Vellefaux, 75010 Paris, France.

[3] Laboratoire de Biochimie Spécialisée, Hotel Dieu, 9 quai Moncousu, 44093 Nantes Cedex 1, France.

*Author for correspondence. Fax 33-1-4249-9247; e-mail thierry.le-bricon@sls.ap-hop-paris.fr.

Received February 12, 2003; accepted August 8, 2003.

Previously published online at DOI: 10.1373/clinchem.2003.017756

[4] Nonstandard abbreviations: AGE, agarose gel electrophoresis; CE, capillary electrophoresis; UV, ultraviolet; CRP, C-reactive protein; and IFE, immunofixation.

sample and reagents, full automation, and high throughput *(8–13)*.

The aim of this study was to evaluate the performance of a recently launched automated multicapillary CE instrument (Capillarys®; Sebia) *(14)* for serum protein analysis. We compared the results with those obtained with our standard semiautomated AGE technique (Hydrasys®-Hyrys®; Sebia) *(13)*.

Materials and Methods

ELECTROPHORESIS

Capillary zone electrophoresis of serum proteins was realized on a Capillarys instrument (4.51 software version; Sebia). The process on the instrument is as follows: Serum specimens (up to 13 racks of 8 primary or secondary sample tubes) are 1:5 diluted with the migration buffer in dilution segments (40 μL of serum to a final volume of 200 μL). Samples are then hydrodynamically injected for 4 s by anodic depression (injected volume, ~1 nL). With the Capillarys β1-β2+® reagent set (Sebia), separation is obtained by applying a voltage of 7 kV for 4 min in eight fused-silica capillaries (total/effective length: 17.5/15.5 cm; i.d., 25 μm) in a pH 10 buffer at 35.5 °C controlled by Peltier effect. Online detection is at 200 nm in an optical cell (i.d., 100 μm) located at the cathodic end of the capillary and connected to the detector by eight optic fibers. In healthy individuals, six main protein fractions are detected in the following order: γ-globulins, β_2-globulins, β_1-globulins, α_2-globulins, α_1-globulins, and albumin (Fig. 1); the electrophoretic mobilities of the predominant individual proteins are indicated (manufacturer's data). Weekly cleaning of the capillaries by a washing solution (enzyme proteolytic surfactant, Capiclean®; Sebia) is recommended.

AGE of serum proteins was performed on a semiautomated Hydrasys system (Sebia) using the Hydragel protein(e) 15/30® reagent set *(13)*. Briefly, after manual sample distribution (10 μL), separation is obtained in an 8 g/L agarose gel with the following automated steps: application for 30 s; separation in Tris-barbital buffer, pH 9.2, at a constant 20 W at 20 °C until 33 V-h have accumulated (~7 min), drying for 10 min at 65 °C, staining with amidoschwarz (4 g/L in an acidic solution), and destaining (0.5 g/L citric acid solution). Total analysis time is 45 min for 30 samples. Densitometric scanning of gels is performed at 570 nm (Hyrys densitometer; Sebia).

Quantitative results for each fraction of the CE or AGE electropherogram are expressed in g/L by multiplying the percentage of total area under the curve by total serum protein concentration.

ANALYTICAL EVALUATION

Capillarys throughput was determined by analyzing 13 × 8 samples (all with bar codes) in a row. Carryover was assessed by alternating serum samples with distilled water (CDM Lavoisier; n = 30). The detection limit and precision studies were conducted according to the guidelines of the French Society of Clinical Biology *(15)*, as follows: the detection limit was defined as 3 SD above the mean of 30 consecutive analyses of a blank (here distilled water), and for imprecision, CVs (within and between runs) were calculated based on 21 runs of at least two control specimens. A normal serum (both quantitatively and qualitatively), one with a low albumin concentration (26.9 g/L), and one containing a well-defined γ-migrating paraprotein (27.7 g/L) were selected. Assay linearity was evaluated by gradually mixing 60 g/L solutions (in 9.0 g/L NaCl solution; Aguettant) of human albumin (Sigma) and γ-globulins (Sigma) to obtain concentrations of 60, 50, 40, 30, 20, 10, and 5 g/L for each fraction (at a constant total protein concentration of 60 g/L). Sensitivity for paraprotein detection was checked by mixing samples containing either a cathodic γ- (5.7 g/L) or anodic γ-monoclonal (6.3 g/L) peak with a normal serum (15.0 g/L polyclonal γ-globulins) down to the lowest peak concentration of 0.1 g/L; this was considered as the highest dilution giving a still discernible band.

We evaluated the effect of storage conditions, using a normal serum specimen kept at room temperature or 4 °C for 1, 8, 24, or 48 h and a lyophilized control (Sebia) stored for 1 week at 4 or 1 month at −20 °C.

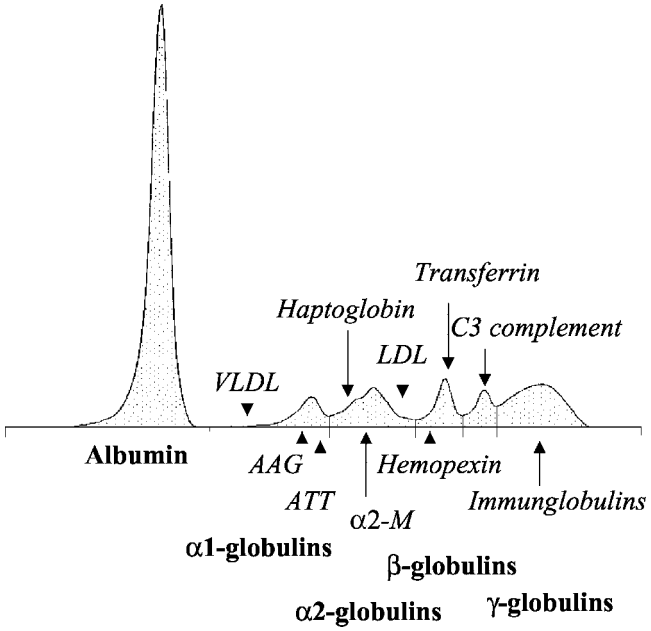

Fig. 1. Capillarys electropherogram of serum proteins from a healthy individual.

The Capillarys automated instrument (β1-β2+ reagent set; Sebia) separates human serum proteins into six main fractions detected at the cathodic end of the capillary in the following order: γ-globulins, β_2-globulins, β_1-globulins, α_2-globulins, α_1-globulins, and albumin. Electrophoretic mobilities of individual proteins were determined by immunosubtraction or overload, depending on the protein (manufacturer's data). Serum samples may display qualitative variations in the α_2- and/or α_1-globulin fraction because of different phenotypes (e.g., for haptoglobin) and the presence of lipoproteins (manufacturer's data). *AAG*, α_1-acid glycoprotein; *ATT*, α_1-antitrypsin; *α2-M*, α_2-macroglobulin.

SPECIMENS AND INTERFERENCE STUDY

A total of 432 blood samples obtained from hospitalized patients were grouped as follows. Group A (n = 265) contained carefully selected sera covering a wide range of electrophoretic patterns: within reference values (n = 25); single well-defined monoclonal immunoglobulin (n = 64; up to 93 g/L) or oligoclonal profiles/weak bands (n = 66); polyclonal hyper-γ-globulinemia (15–85 g/L; n = 30), hypo-γ-globulinemia (0.5–6.0 g/L; n = 30), hypoalbuminemia (10–30 g/L; n = 25), hyper-α_1-globulinemia (>3 g/L) or hyper-α_2-globulinemia (>9 g/L; n = 25). Group B (n = 98) contained unselected specimens from our daily routine that were analyzed blindly by AGE and CE. Group C (n = 69) contained samples with potential interference, including hemolysis (n = 19), lactescence (triglycerides >10 mmol/L; n = 7), icterus (total bilirubin >50 μmol/L; n = 20), increased C-reactive protein (CRP; >100 mg/L; n = 9), fibrinogen (sodium heparinate plasma; n = 5), and cryoglobulins (n = 9).

Blood was collected on a 7-mL tube without anticoagulant (Becton Dickinson) and centrifuged for 15 min at 1500*g* at 20 °C. Total serum protein was measured and AGE performed within 8 h before storage at −20 °C (groups A and C) or was analyzed immediately by CE (group B). Cryoglobulin samples were run on the Capillarys after 24 h of storage at 4 or 37 °C.

We evaluated the effects of UV-absorbing substances on the CE electropherogram by adding to 90 μL of a normal serum, 10 μL of a 70 g/L hemoglobin solution prepared from hemolysates *(16)*, a sodium and meglumine ioxitalamate contrast agent (Telebrix®; Guerbet), or a lipid emulsion for parenteral nutrition (Ivelip® 10%; Baxter). We also evaluated the effect of a gelatin-based plasma substitute (Plasmion®; Roger Bellon) by mixing 50 μL of the solution with 50 μL of normal serum.

This study was conducted in accordance with the requirements of the Helsinki Declaration of 1975 as revised in 1996.

OTHER BIOCHEMICAL MARKERS

Total serum protein was determined by the Biuret reaction on a Modular® system (Roche). Bilirubin, triglyceride, and CRP concentrations were determined by standard biochemical methods using Roche reagent sets (Modular); hemoglobin was measured on a BN-100® nephelometer (Dade-Behring).

In case of discordant results between CE and AGE for group A patients, we performed serum immunofixation (IFE) using the Hydragel 4 IF® reagent set (Hydrasys; Sebia) with antibodies against IgG, IgA, IgM, and κ and λ light chains.

STATISTICS

Statistical analysis was performed with Sigmastat®, Ver. 2.0 (Jandel Scientific). Results are expressed as the median and range or the mean and SD according to the population distribution (Kolmogorov–Smirnov test). Linear regression was used for analytical evaluation of CE linearity, and Deming regression analysis *(17)* was used for comparison of CE and AGE. Statistical significance was set at $P < 0.05$ for all tests.

Results

ANALYTICAL PERFORMANCES

Measured throughput was 60 samples/h with no sample carryover (median absorbance, 0.003, as for signal noise). In the precision study, maximum absorbance (median values) ranged from 0.1722 (low albumin control) to 0.2407 (paraprotein control) with CVs between 9.3% and 14% (between-run data). The results obtained for different protein fractions are presented in Table 1. The assay was linear over a concentration range of 60–10 g/L for albumin ($r = 0.99$) and γ-globulins ($r = 0.98$), with the detection limit (absorbance of 0.010) corresponding to ~3.5 g/L of a mixture containing 5 parts albumin and 1 part γ-globulin. The detection limit was 0.5 g/L for the anodic γ-migrating paraprotein and 0.7 g/L for the more cathodic γ-migrating paraprotein. Storage conditions had no significant impact (both quantitative and qualitative) on the Capillarys electropherogram (data not shown).

METHOD COMPARISON STUDY

The results of the method comparison for protein fraction quantification in the subgroup of group A patients without paraprotein (n = 116) are presented in Table 2. Differences between methods were proportional to the protein fraction concentration for albumin ($r = 0.52$) and α_1-globulins ($r = 0.34$; linear regression, $P < 0.001$); we observed no significant difference between methods for

Table 1. Result of precision study for the Capillarys, using three controls.[a]

Fraction	Normal (n = 21)	Low albumin (n = 21)	Paraprotein (n = 21)
Albumin			
Median, g/L	40.9	24.9	20.4
Within-/between-run CVs, %	1.2/2.1	1.4/1.4	2.6/3.4
α_1-Globulins			
Median, g/L	6.6	3.8	11.4
Within-/between-run CVs, %	4.8/11	7.1/10	2.7/5.3
α_2-Globulins			
Median, g/L	8.5	3.2	5.0
Within-/between-run CVs, %	2.8/3.8	2.9/4.0	3.4/3.5
β1-Globulins			
Median, g/L	4.7	2.4	2.4
Within-/between-run CVs, %	2.8/3.7	2.9/4.0	6.1/7.0
β_2-Globulins			
Median, g/L	2.5	1.7	3.5
Within-/between-run CVs, %	4.7/5.7	4.5/7.0	7.3/7.1
γ-Globulins			
Median, g/L	7.9	6.0	28.3
Within-/between-run CVs, %	2.7/3.5	2.0/5.7	1.5/3.3

[a] Total protein: normal control, 71 g/L; low albumin, 42 g/L; paraprotein, 70 g/L.

Table 2. Method comparison for protein fraction quantification in patients without paraprotein.

	Capillarys vs Hydrasys[a]	
Fraction	Deming regression analysis (n = 116)	Median difference (95% confidence interval), g/L
Albumin	r = 0.99; slope = 0.91 (0.01); intercept = −1.9 (0.3)	−5.4[b] (−2.7 to −8.1)
α_1-Globulins	r = 0.84; slope = 1.26 (0.10); intercept = 3.3 (0.2)	4.0[b] (2.5–5.4)
α_2-Globulins	r = 0.94; slope = 1.33 (0.08); intercept = −2.0 (0.6)	0.7[b] (−0.9 to 2.2)
β-Globulins	r = 0.82; slope = 1.36 (0.08); intercept = −1.6 (0.5)	0.6[b] (−1.0 to 2.8)
γ-Globulins	r = 0.99; slope = 1.02 (0.01); intercept = −0.15 (0.05)	−0.1 (−2.5 to 1.7)

[a] SD for slope and intercept in parentheses.
[b] P <0.001 (RM-ANOVA).

γ-globulins (Table 2). In the paraprotein subgroup, the following results were observed for γ-migrating paraprotein quantification (n = 69): mean (SD) slope, 0.96 (0.04); mean (SD) intercept, 1.4 (0.2); r = 0.99; median difference, 1.2 g/L by CE (P <0.001). A Bland–Altman plot of individual differences is provided in Fig. 2.

The total number of samples with at least one detectable abnormal peak in the α_2 to the γ zone was 135 by CE and 130 by AGE (Fig. 3). There was a 100% agreement in the subgroup of patients with a single well-defined paraprotein by AGE (n = 64). In others subgroups, additional peaks detected by CE corresponded to small or faint bands identified as monoclonal by IFE in 70% of cases. Fewer peaks could be quantified by CE than AGE because 15 of them (all quantified by AGE) were shifted from the γ zone (by AGE) to the β_2 zone (identified as IgG in 58%,

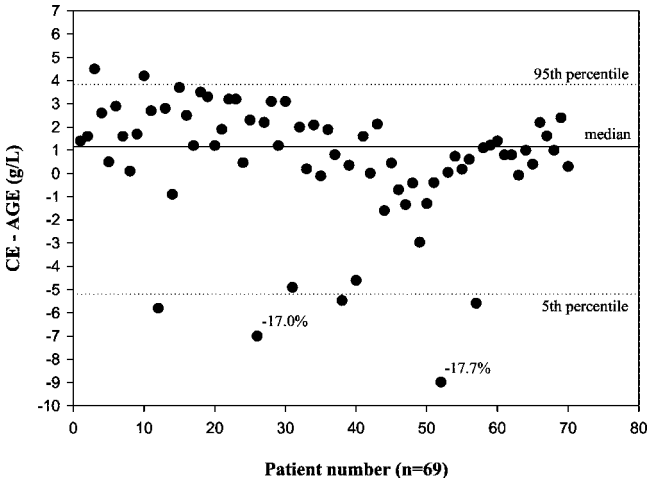

Fig. 2. Bland–Altman analysis of paraprotein quantification by CE and AGE.

For paraproteins quantified by both CE and AGE (n = 69), the correlation between the two techniques was significant (r = 0.99). The median difference between CE and AGE was +1.2 g/L (P <0.001).

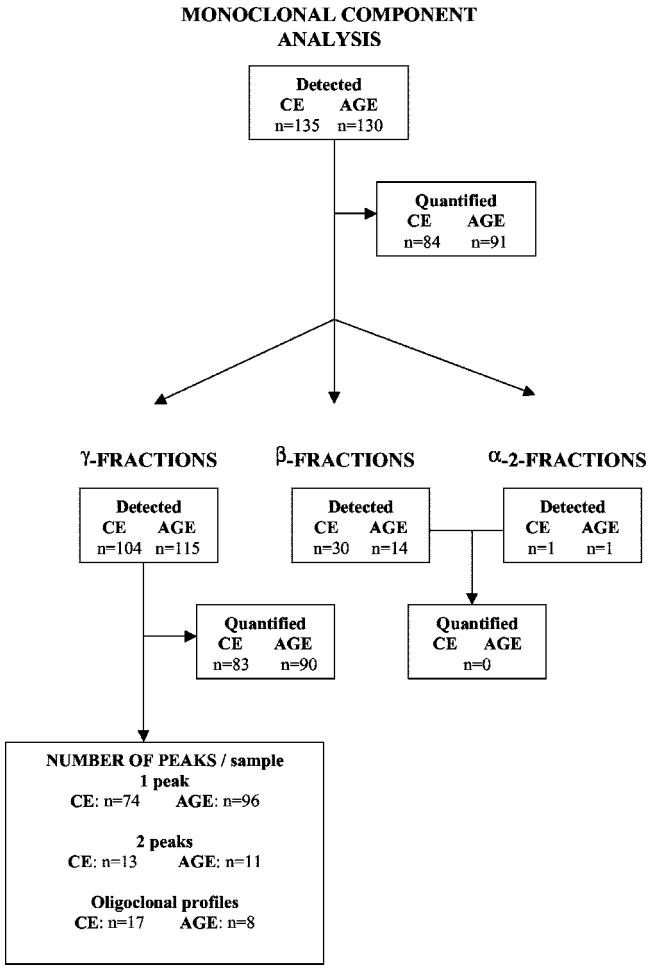

Fig. 3. Paraprotein detection and quantification by CE and AGE.

Selected sera (n = 265; group A) covering a wide range of electrophoretic patterns were analyzed by the Capillarys and AGE [Hydrasys-Hyrys; Hydragel protein(e) 15/30 reagent set; Sebia], including 130 samples with a single monoclonal immunoglobulin (γ-migrating, n = 115; β-migrating, n = 14; α_2-migrating, n = 1) or an oligoclonal profile (two or more peaks by AGE).

IgM in 34%, IgA in 8%; κ in 58%, and λ in 42%). Five patients with hypo-γ-globulinemia by AGE had a small detectable peak by CE; only the three quantitatively most important peaks (0.7, 0.9, and 2.1 g/L) were detected and identified by IFE as oligoclonal, IgGκ, and free κ or IgDκ or IgEκ, respectively. In group B, results for γ-globulins (CE/AGE; n = 98) were 62 and 71 normal, respectively; 17 and 15 with a single well-defined quantified peak; and 19 and 12, respectively, with a small peak or an oligoclonal profile.

IN VIVO (GROUP C) AND IN VITRO INTERFERENCES

Hemolyzed serum samples had increased β_1-globulins and/or α_2-globulins. Patients with a CRP <240 mg/L (n = 4) had no evident modification of the γ zone of the electropherogram; a faint band in the anodic region of the γ-globulins (close to fibrinogen) was observed in the two specimens with concentrations >300 mg/L. Icteric samples had no apparent modification of the CE electrophero-

gram, whereas lipemic samples had increased α_1-globulins with, in some cases, an additional peak close to albumin. After cryoglobulin storage at 4 °C, no peak was detected in the γ zone of the Capillarys electropherogram in seven of nine samples. At 37 °C, a peak was detected in 100% of cases that corresponded to the measured cryoprecipitate (Table 3). Free hemoglobin, Telebrix, and Ivelip were responsible for the appearance of additional peaks in the β_1 zone (hemoglobin), at the junction of the α_1-/α_2-globulin zones (Telebrix), or in the α_1-globulin zone (Ivelip). Addition of Plasmion increased the γ-globulin fraction in a polyclonal-like way, a phenomenon confirmed in vivo in a patient treated with a similar product (Gélofusine®; B Braun Médical). Figures showing the interference of selected compounds (lipids, cryoglobulins, contrast agent, plasma substitute, and fibrinogen) are available as a Data Supplement accompanying the online version of this article at http://www.clinchem.org/content/vol49/issue11/.

Discussion

The Capillarys is the second generation of automated multicapillary instruments for serum protein analysis. Because it is fully automated (bar-code identification for patients and racks, preseparation steps, direct postseparation quantification), it requires almost no technical manipulation. The reproducibility of the Capillarys assay was excellent from sample preseparation steps (CV ~10% for sampling, dilution, and injection) to electrophoretic separation and quantification of protein fractions (CV, 1.2–7.1%), except for the flat, often large and heterogeneous α_1-globulin zone (CV >10% with irreproducible integration). Similar results have been published with the Paragon CZE2000 *(8, 9)* and the earlier 4.41 version of the Capillarys *(14)*. Despite good correlation with the Hydrasys-Hyrys for albumin and γ-globulins (r = 0.99), there was an ~200% positive difference vs AGE for α_1-globulins and a 15% negative difference for albumin. Reports in the literature indicate similar results for the Paragon CZE2000 (~100% difference for α_1-globulins, but lower difference for albumin) *(8, 9)* and the Capillarys *(14)*, highlighting the need for an adjustment of the protein fraction reference interval *(9, 18)*. Comparisons with immunonephelometry and colorimetry suggest that quantification of albumin and α_1-globulin in the low-UV range is more accurate than protein staining *(8, 9)*, especially for α_1-acid glycoprotein because of its high sialic acid content. Overall, the throughput for the Capillarys (~60 samples/h) was better than for AGE (~30 samples/h with the Hydrasys-Hyrys) *(13)* and the Paragon CZE2000 (~40 samples/h) *(8)*.

Table 3. Analysis of cryoglobulins by the Capillarys system after storage at 4 and 37 °C.[a]

Patient no.	Cryoglobulin type	Cryocrit, %	Peak at 4 °C, % of AUC[b]	Peak at 37 °C, % of AUC
1	Untyped	1	No	Small peak
2	III	1	Oligoclonal (n = 2)	Oligoclonal (n = 3)
3	II (IgMκ)	18	3.1	20.3
4	I (IgGκ)	3	No	5.7
5	II (IgMκ)	5	No	5
6	II (IgMκ)	3	No	Small peak
7	Untyped (RF+)	1	No	Small peak
8	Untyped (IgGκ)	1	No	3.7
9	Untyped	1	No	Small peak

[a] Samples were stored at 4 or 37 °C for 24 h before analysis by CE.
[b] AUC, area under the curve; RF, rheumatoid factor.

The detection limits for paraproteins in the Capillarys were in the 0.5 g/L range, similar to the detection limits reported for the Paragon CZE2000 *(8)* and for the semiautomated Hydrasys-Hysis AGE system *(13)*. Importantly, there were no well-defined single paraproteins missed by CE, as reported for a few cases with the earlier 4.41 buffer and software version of the Capillarys *(14)* or the Paragon CZE2000 *(19, 20)*. In oligoclonal, hypo- and hyper-γ-globulinemic subgroups, the Capillarys displayed higher sensitivity than AGE for the detection of weak monoclonal bands (70% of positivity by IFE), as has been observed with the Paragon CZE2000 *(20)*. In 30% of discordant results with AGE (difficult and/or complex profiles of the γ-globulins), however, abnormalities detected by CE were not confirmed by IFE, the gold standard method. They might correspond to other anodic γ-proteins, such as small amounts of fibrinogen, CRP, or degraded products of the C_3 complement *(14)*. Because degradation of the C_3 fraction of the complement is a temperature-dependent phenomenon *(14)*, serum storage at 4 °C for 48 h or at −20 °C for more prolonged periods is recommended. Concerning paraprotein quantification, there was no gain in the number of peaks quantified by CE because of γ- to β_2-migration shifts observed in 11.5% of paraproteins (mostly IgM or IgG). Importantly, however, the median difference with AGE was limited to 1.2 g/L with only five outliers (maximum difference, −9.0 g/L, or −17%), an improvement over previously published CE methods *(9, 19)*.

Hemolysis, icterus, and turbidity (and associations of these) represent the most frequently encountered serum interferences in clinical chemistry. The electrophoretic mobility of hemoglobin in the Capillarys was similar to that reported with the Hydrasys-Hyrys *(21)*, the Paragon CZE2000 *(8, 9)*, and the earlier, 4.41 version of the Capillarys *(14)*: α_2-globulins (haptoglobin-hemoglobin complex) and β-globulins for free hemoglobin. Icterus had no effect on the CE electropherogram (up to 300 μmol/L total bilirubin), but an increase in the baseline or, sometimes, a well-defined VLDL peak appeared between albumin and other α_1-globulins proteins in hyperlipemic samples. An optional computer-assisted correction is available in the present 4.51 version, which deletes the area under the curve between albumin and α_1-globulins. Use of this correction improved the automated integration of α_1-globulins (~38% improvement of the analytical

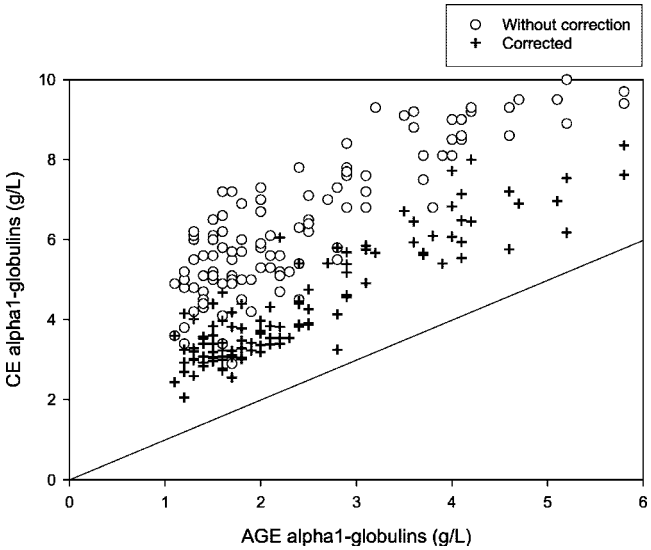

Fig. 4. Correlation between CE and AGE for α_1-globulins.

With the Capillarys $\beta 1$-$\beta 2+$ reagent set, VLDL migrates between albumin and α_1-globulins, producing an increase in the baseline or, in some cases, an additional α_1 peak. Computer-assisted correction of the α_1 zone efficiently reduced the difference with quantification by AGE (median, 1.8 vs 4.0 g/L before correction) in the subgroup of patients without paraprotein (n = 116).

precision) and the correlation between CE and AGE [r = 0.90; slope = 1.29 (0.08); intercept = 1.2 (0.2)] and reduced the difference with AGE to +1.8 g/L (Fig. 4). After adjustment of reference values for α_1-globulins, this correction could allow an easier screening of α_1-antitrypsin deficiencies by CE *(22)*. A computer-supported algorithm has also been developed by Beckman for the Paragon CZE2000 to recognize deficiency variants of α_1-antitrypsin *(23)*. Fibrinogen (heparinized samples or incompletely clotted serum) migrates in the anodic part of the γ-globulins, close to C_3. The high sensitivity of CE might also lead to the detection of endogenous proteins present in low concentrations (undetected by AGE), as observed in two specimens with a CRP >300 mg/L (faint band in the anodic γ-globulin zone). The clinical incidence of this phenomenon might be limited because, in our experience, only ~0.5% of hospitalized patients have a serum CRP >300 mg/L, and it rarely exceeds 500 mg/L. Interestingly, controlling the temperature of the capillary at 35.5 °C also prevented cryoglobulins from precipitating during electrophoretic migration, thus allowing their detection and accurate quantification, an advantage over AGE.

Overcoming interference by exogenous UV-absorbing substances is a new challenge in CE, not present in standard gel-based methods with indirect quantification by dye binding. As observed previously *(14, 24)*, an iodinated radioopaque agent (Telebrix) was detected in the form of a α_1-/α_2-monoclonal-like peak, leading to a large converse error for all other fractions. During intravenous infusion of lipid emulsions (parenteral nutrition), the Capillarys algorithm should efficiently correct the α_1-globulin zone. We also established that gelatin-based plasma substitutes falsely increase γ-globulins in a polyclonal-like way. Visual inspection of serum specimens and knowledge of patient treatments are, therefore, of primary importance to avoid gross quantitative errors and misleading interpretations. Centrifugation (high speed or ultracentrifugation) for lipids or dialysis for small molecules might also help reduce interference. Considering the short half-lives of these drugs (e.g., 4 h for Gélofusine), performing CE of serum proteins at a distance from drug administration might be the safest alternative.

In conclusion, the Capillarys is an automated multicapillary zone electrophoresis instrument designed for human serum protein analysis. This high-throughput analytical system can save a substantial amount of technical time compared with AGE methods. Although not currently available (but available on the Paragon CZE2000), the most interesting perspective of the Capillarys is the immunologic identification of monoclonal proteins *(25–27)*. Analysis of low-protein biological fluids (e.g., cerebrospinal fluid and urine) remains difficult *(28, 29)* because of the relative low sensitivity of UV detection and the presence of UV-absorbing interfering substances (especially in urine). More specialized analyses, such as for hemoglobin A_{1C} *(30)* or transferrin sialoforms *(31)*, have been successfully developed on research monocapillary electrophoresis systems, but are not currently available on multicapillary electrophoresis systems.

References

1. Attaelmannan M, Levinson SS. Understanding and identifying monoclonal gammopathies. Clin Chem 2000;46:1230–8.
2. Keren DF, Warren JS, Lowe JB. Strategy to diagnose monoclonal gammopathies in serum: high resolution electrophoresis, immunofixation, and κ/λ quantitation. Clin Chem 1988;34:2196–204.
3. Lehmann R, Voelter W, Liebich HM. Capillary electrophoresis in clinical chemistry. J Chromatogr B 1997;697:3–35.
4. Jenkins MA, Guerin MD. Capillary electrophoresis as a clinical tool. J Chromatogr B 1996;682:23–34.
5. Lauer HH, McManigill D. Capillary zone electrophoresis of proteins in untreated fused silica tubing. Anal Chem 1986;58:166–9.
6. Chen FA. High-resolution protein analysis by automated capillary electrophoresis. Clin Chem 1992;38:1651–3.
7. Chen FA. Rapid protein analysis by capillary electrophoresis. J Chromatogr 1991;559:69–78.
8. Bienvenu J, Graziani MS, Arpin F, Bernon H, Blessum C, Marchetti C, et al. Multicenter evaluation of the Paragon CZE 2000® capillary zone electrophoresis system for serum protein electrophoresis and monoclonal component typing. Clin Chem 1998;44:599–605.
9. Bossuyt X, Schiettekatte G, Bogaerts A, Blanckaert N. Serum protein electrophoresis by CZE 2000 clinical capillary electrophoresis system. Clin Chem 1998;44:749–59.
10. Meunier JC. La détection et la caractérisation des composés monoclonaux difficiles dans le sérum : techniques sur gel ou électrophorèse capillaire. Ann Biol Clin 1999;57:605–10.
11. Jaeggi-Groisman SE, Byland C, Gerber H. Improved sensitivity of

capillary electrophoresis for detection of bisalbuminemia. Clin Chem 2000;46:882–3.

12. Kalambokis G, Kitsanou M, Kalogera C, Kolios G, Seferiadis K, Tsianos E. Inherited bisalbuminemia with benign monoclonal gammopathy detected by capillary but not agarose gel electrophoresis. Clin Chem 2002;48:2076–7.

13. Godey F, Ropert M, Bouasria A, Lucas-Clerc C, Guenet L, Savoure N, et al. Le Paragon CZE 2000® et l'Hydrasys® pour l'électrophorèse des protéines sériques et le typage des immunoglobulines monoclonales sériques. Ann Biol Clin 1999;57:337–44.

14. Bossuyt X, Lissoir B, Mariën G, Maisin D, Vunckx J, Blanckaert N, et al. Automated serum protein electrophoresis by Capillarys®. Clin Chem Lab Med 2003;41:704–10.

15. Vassault A, Grafmeyer D, de Graeve J, Cohen R, Beaudonnet A, Bienvenu J. Analyses de biologie médicale: spécifications et normes d'acceptabilité à l'usage de la validation de techniques. Ann Biol Clin 1999;57:685–95.

16. Jay DW, Provasek D. Characterization and mathematical correction of hemolysis interference in selected Hitachi 717 Assays. Clin Chem 1993;39:1804–10.

17. Martin RF. General Deming regression for estimating systematic bias and its confidence interval in method-comparison studies. Clin Chem 2000;46:100–4.

18. Petrini C, Alessio MG, Scapellato L, Brambilla S, Franzini C. Serum proteins by capillary zone electrophoresis: approaches to the definition of reference values. Clin Chem Lab Med 1999;37:975–80.

19. Jenkins MA, Kulinskaya E, Martin HD, Guerin MD. Evaluation of serum protein separation by capillary electrophoresis: prospective analysis of 1000 specimens. J Chromatogr B 1995;672:241–51.

20. Henskens Y, De Winter J, Pekelharing M, Ponjee G. Detection and identification of monoclonal gammapathies by capillary electrophoresis. Clin Chem 1998;44:1184–90.

21. Benlakehal M, Le Bricon T, Feugeas JP, Bousquet B. Influence de l'hémolyse sur le dosage et l'électrophorèse des protéines sériques. Ann Biol Clin 2000;58:367–71.

22. Gonzales-Sagrado M, Lopez-Hernandez S, Martin-Gil FJ, Tasende J, Banuelos MC, Fernandez-Garcia N, et al. α_1-Antitrypsin deficiencies masked by a clinical capillary electrophoresis system (CZE 2000). Clin Biochem 2000;33:79–80.

23. Jonsson M, Carlson J. Computer-supported interpretation of protein profiles after capillary electrophoresis. Clin Chem 2002;48:1084–93.

24. Arranz-Pena ML, Gonzalez-Sagrado M, Olmos-Linares AM, Fernandez-Garcia N, Martin-Gil FJ. Interference of iodinated contrast media in serum capillary zone electrophoresis. Clin Chem 2000;46:736–7.

25. Bossuyt X, Bogaerts A, Schiettekatte G, Blanckaert N. Detection and classification of paraproteins by capillary immunofixation/subtraction. Clin Chem 1998;44:760–4.

26. Litwin CM, Anderson SK, Philipps G, Martins TB, Jaskowski TD, Hill HR. Comparison of capillary zone and immunosubstraction with agarose gel and immunofixation electrophoresis for detecting and identifying monoclonal gammopathies. Am J Clin Pathol 1999;112:411–7.

27. Jonsson M, Carlson J, Jeppson JO, Simonsson P. Computer-supported detection of M-components and evaluation of immunoglobulins after capillary electrophoresis. Clin Chem 2001;47:110–7.

28. Kolios G, Bairaktari E, Tsolas O, Seferiadis K. Routine differential diagnosis of proteinurias by capillary electrophoresis. Clin Chem Lab Med 2001;39:784–8.

29. Sanders E, Katzmann JA, Clark R, Oda RP, Shihabi Z, Landers J. Development of capillary electrophoresis as an alternative to high resolution agarose electrophoresis for the diagnosis of multiple sclerosis. Clin Chem Lab Med 1998;37:37–45.

30. Doelman CJA, Siebelder CWM, Nijhof WA, Weykamp CW, Janssens J, Penders TJ. Capillary electrophoresis system for hemoglobin A1c determinations evaluated. Clin Chem 1997;43:644–8.

31. Giordano BC, Muza M, Trout A, Landers JP. Dynamically-coated capillaries allow for capillary electrophoretic resolution of transferrin sialoforms via direct analysis of human serum. J Chromatogr B 2000;742:79–89.