

Performance of the Sebia CAPILLARYS 2 for Detection and Immunotyping of Serum Monoclonal Paraproteins

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Abstract

We evaluated the performance of the CAPILLARYS 2 (Sebia, Norcross, GA) capillary electrophoresis system for detection and identification of monoclonal proteins in serum samples. We analyzed 104 serum specimens by Sebia Hydragel serum protein electrophoresis (agarose gel electrophoresis [AGE]/immunofixation electrophoresis [IFE]) and CAPILLARYS 2 capillary zone electrophoresis (CZE)/immunofixation. AGE and CZE had sensitivities of 90% and 81%, respectively, based on IFE as the “gold standard,” and all bands detected were confirmed by IFE (100% specificity). AGE and CZE had an overall agreement of 91% on serum protein electrophoresis. In the population tested, IgG was detected in 29% of samples by IFE and 30% using immunofixation. Similarly IgA was detected in 9% of cases by IFE and 8% by immunofixation. IgM and light chains were detected in 6% and 3% of cases, respectively, by IFE, whereas CZE/immunofixation did not detect any IgM or light chains. In our hands, AGE and CZE had the same specificity for detection of monoclonal proteins; however, CZE/immunofixation seems to be less sensitive than IFE for the detection of IgM and, possibly, serum light chains.

Plasma cell myeloma is a common lymphoid malignancy, accounting for 15% of all hematologic malignancies in the United States.¹ Quantifying and immunotyping of paraproteins has an important role in the diagnosis, classification, and management of this disorder. In addition, a premalignant lesion, monoclonal gammopathy of undetermined significance (MGUS), is usually diagnosed and followed up by serum protein electrophoresis. Currently, the method of choice for detection of serum or urine paraprotein is protein electrophoresis on agarose gel (AGE). Serum proteins are separated into 6 regions: albumin, α_1 , α_2 , β_1 , β_2 , and γ . The gel is stained with amido black or another protein stain, and the concentration of each band is quantified by a densitometer. Paraprotein is usually shown as a sharp peak in the γ region. Immunotypes are determined by immunofixation electrophoresis (IFE) in which specific antibodies are overlaid after electrophoresis and the corresponding immunoglobulin (IgG, IgA, IgM, κ , and λ) is bound and visualized by acid violet or another protein stain.

Capillary zone electrophoresis (CZE) has emerged as a powerful technique. The technique achieves serum protein separation into the same 6 regions as AGE but does so without using gels. Rather separation is accomplished in a liquid buffer system running through parallel, narrow-bored capillaries consisting of fused silica. The narrow-bored capillaries permit the use of very high voltage. Details of the CZE technique have been reviewed recently.^{2,3} The sample runs through the narrow capillary tube, and direct protein detection is achieved by a measurement at 200 nm, eliminating the need for staining while improving accuracy and linearity. This method has the advantages of high efficiency (multiple samples can be run in parallel), and the system is fully automated.

CZE can be combined with immunosubtraction to type the immunoglobulins. The sample is treated with specific anti-serum to precipitate and remove the corresponding immunoglobulin. The specific immunotype is shown as a reduction of the peak on CZE.

Studies have shown that the Beckman Paragon 2000 capillary electrophoresis system (Beckman, Brea, CA) is a good alternative for AGE and IFE,^{4,5} and the Sebia CAPILLARYS capillary electrophoresis systems (Sebia, Norcross, GA) have reliable performance for paraprotein identification and quantification.⁶ Nevertheless, some reports indicate that CZE may be more sensitive but less specific than AGE at detecting paraproteins.⁷⁻⁹ This could translate into performing unnecessary and costly IFE or immunotyping testing. In addition, conflicting results and failure to identify certain paraproteins have been reported.^{3,10} Immunosubtraction is available on the CAPILLARYS 1 and CAPILLARYS 2 systems. In this study, we compared the performance of the CAPILLARYS 2 system with that of the HYDRASYS system (Sebia) for the detection of paraproteins and immunotyping.

Materials and Methods

Agarose Gel Electrophoresis

Protein electrophoresis was performed using the semiautomated Sebia HYDRASYS system with the Hydragel protein 15/30 reagent set (Sebia) according to the manufacturer's instructions. Proteins were stained with amido black, and densitometric scanning was performed at 570 nm (HYRYS densitometer, Sebia). Immunofixation was also performed using the Sebia HYDRASYS system according to the manufacturer's instructions using fixative or monospecific antisera for IgG, IgA, IgM, κ , or λ and was considered the "gold standard" as to whether there was a band and the identity of the bands.

CZE and Immunosubtraction

CZE of serum proteins was performed on a CAPILLARYS 2 instrument (5.07 software version) according to the manufacturer's instructions. This procedure has been described previously.⁶

Patient Specimens and Electrophoresis Interpretation

We selected 104 serum samples received by the special chemistry laboratory at University of Alabama at Birmingham Hospital in 2 consecutive months as a subset of all samples received for this study. Among the 104 serum samples, 47 were from males and 57 from females. Race distributions of the patients were as follows: white, 68; black, 28; other, 2; and unknown, 6. The median age was 60 years (range, 16-88 years).

AGE/IFE and CZE/immunosubtraction were performed for each sample. The results were independently interpreted by 8 qualified reviewers (3 attending faculty [R.W.H., R.G.L., and C.A.R.], 2 experienced medical technologists [B.T. and P.L.E.], and 3 pathology residents [Z.Y., K.H., and Y.A.P.]). A simple majority of at least 5 of 8 was necessary to assign the sample to a specific category. CAPILLARYS 2 immunosubtraction studies were performed before the commercial release of the immunosubtraction product. This was a blinded study in which the readers knew only the results of the test method they were evaluating at that time. For example, they did not know the AGE, CZE, or immunosubtraction results when assessing the IFE results. Because the study was blinded, all samples were run 1 time using the default or standard dilution for all tests. In addition, there was no patient information available to the readers when assessing test results. In the follow-up study, IgM was quantitated by rate nephelometry using a Beckman-Coulter Array protein system (Beckman) and interpreted by 1 reviewer (Z.Y.). The clinical diagnoses were based on a retrospective review of electronic medical records. This study was approved by the institutional review board.

Statistical Analysis

The categorical assignments between AGE and CZE were analyzed with the Fisher exact test. Using IFE as the gold standard, the sensitivity, specificity, and positive and negative predictive values of CZE and AGE were calculated. The exact binomial confidence intervals were computed for each estimate. Diagnostic test accuracy was evaluated by using the κ statistic.

Results

Agreement Between AGE and CZE

For the 104 specimens, 43 (41.3%) vs 39 (37.5%) were found to have a "band," 59 (56.7%) vs 62 (59.6%) had "no band," and 2 (1.9%) vs 3 (2.9%) were inconclusive for AGE vs CZE, respectively (Table 1). The overall concordance of the 2 methods for band and no-band classification was 91%.

Similarly 36 (84%) vs 35 (90%) band and 45 (76%) vs 47 (76%) no-band cases received consensus agreement for AGE and CZE, respectively. With band and no-band cases added together, a total of 81 cases (77.9% of all cases) vs 82 (78.8%) received consensus determination by AGE and CZE, respectively. Majority agreement was reached in 102 cases (98.1%) for AGE compared with 101 cases (97.1%) for CZE. There was no significant difference in majority or consensus band-no-band designations between AGE and CZE ($P > .25$; Fisher exact test).

Agreement of AGE and CZE With IFE

In the population tested, IgG was detected in 29% of patient samples by IFE compared with 30% using immunosubtraction. Similarly IgA was detected in 9% of cases by IFE compared with 8% using CZE/immunosubtraction. IgM and light chains were detected in 6% and 3% of cases, respectively, by IFE, whereas CZE/immunosubtraction did not detect any IgM or light chains. Data presented in **Table 2** and **Table 3** indicate that CZE and AGE had sensitivities of 81% and 90%, specificities of 100% and 100%, positive predictive values of 100% and 100%, negative predictive values of 85% and 92%, and accuracy values of 91% and 95%, respectively.

Based on IFE being the gold standard for immunotyping, CZE/immunosubtraction had 7 (18%) discrepant results out of 39 band cases detected by AGE and CZE **Table 4**. Four of the cases involved IgM, and 3 of the 4 cases involved 2 bands in which IgM was missed by CZE/immunosubtraction; the fourth case, IgMλ, was mistyped as IgGκ by CZE/immunosubtraction. One had 2 bands where the free light chain was missed by CZE/immunosubtraction, and 2 were IgAs, one having 2 IgA bands by IFE and CZE/immunosubtraction typed a single IgA band, and the second having a single IgA band by IFE and 2 IgA bands by CZE/immunosubtraction. Overall, IFE detected 6 cases involving IgM that were not detected by CZE/immunosubtraction. **Figure 1** demonstrates a representative IgM case.

To further investigate the apparent inability of the CZE/immunosubtraction to detect low-level IgM monoclonal proteins, we collected 5 additional samples that were positive for a single IgM band based on AGE/IFE. All 5 samples produced a detectable IgM band by CZE/immunosubtraction when examined by a single reviewer (Z.Y.). These samples were not from the same cases originally missed by CZE/immunosubtraction, and the IgM concentrations for these samples ranged from 545 to 4,310 mg/dL (5.45-43.10 g/L) as determined by rate nephelometry. The IgM monoclonal protein concentrations, as determined by AGE of the samples not identified by CZE/immunosubtraction in **Table 4**, seem to be less than the concentrations of the 5 samples collected subsequently.

CZE also missed 2 free light chain cases identified by AGE. Both had free κ light chain identified by IFE, and the cases were diagnosed as multiple myeloma. A representative sample is shown in **Figure 2**.

Unresolved Cases

There were also 5 (12%) of 43 cases on AGE/IFE and 6 (15%) of 39 cases on CZE/immunosubtraction for which a monoclonal band was detected but no agreement was reached regarding the immunotype. Most of them were multiple myeloma cases that may represent posttreatment effects or aberrant proliferation of plasma cells. The remaining 3 cases were 1 each of autoimmune disease, renal failure, and breast

Table 1
Summary of “Band” or “No Band” in 104 Specimens Tested for Monoclonal Proteins*

	Agarose Gel Electrophoresis	Capillary Zone Electrophoresis
No band	59 (56.7)	62 (59.6)
Band	43 (41.3)	39 (37.5)
Inconclusive	2 (1.9)	3 (2.9)
Total	104	104
Consensus of reviewers (8/8)	81 (77.9)	82 (78.8)
Majority of reviewers (5/8)	102 (98.1)	101 (97.1)

* Data are given as number (percentage). The consensus and majority data reflect review by 8 experienced reviewers.

Table 2
CZE vs IFE: Classification of “Band” or “No Band” in Specimens Tested for Monoclonal Proteins*

	IFE		
	Band	No Band	Total
CZE			
Band	39	0	39 (100)
No band	9	53	62 (85)
Total	48 (81)	53 (100)	101 (91)

CZE, capillary zone electrophoresis; IFE, immunofixation electrophoresis.

* Totals are given as number (percentage), and percentages indicate the number of specimens correctly identified based on IFE as the gold standard, and samples with disagreement (ie, 4 agree and 4 disagree) are not included.

Table 3
AGE vs IFE: Classification of “Band” or “No Band” in Specimens Tested for Monoclonal Proteins*

	IFE		
	Band	No Band	Total
AGE			
Band	43	0	43 (100)
No band	5	54	59 (92)
Total	48 (90)	54 (100)	102 (95)

AGE, agarose gel electrophoresis; IFE, immunofixation electrophoresis.

* Totals are given as number (percentage), and percentages indicate the number of specimens correctly identified based on IFE as the gold standard, and samples with disagreement (ie, 4 agree and 4 disagree) are not included.

Table 4
Disagreement on Immunotype Between IFE and Immunosubtraction in Specimens Tested for Monoclonal Proteins

Case No.	IFE	Monoclonal Protein (mg/dL)*	Immunosubtraction	Diagnosis
1	IgGκ, IgMλ	333	IgGκ	MM
2	IgMλ	290	IgGκ	MM
3	IgGλ, IgMκ	NA	IgGλ	MM
4	IgGκ, free κ light chain	2,670	IgGκ	MM
5	IgGλ, IgMλ	490	IgGλ	MM
6	2 IgAλ	NA	IgAλ	MM
7	IgAκ	NA	2 IgAκ	MM

IFE, immunofixation electrophoresis; MM, multiple myeloma; NA, not available.

* The concentration was determined by agarose gel electrophoresis.

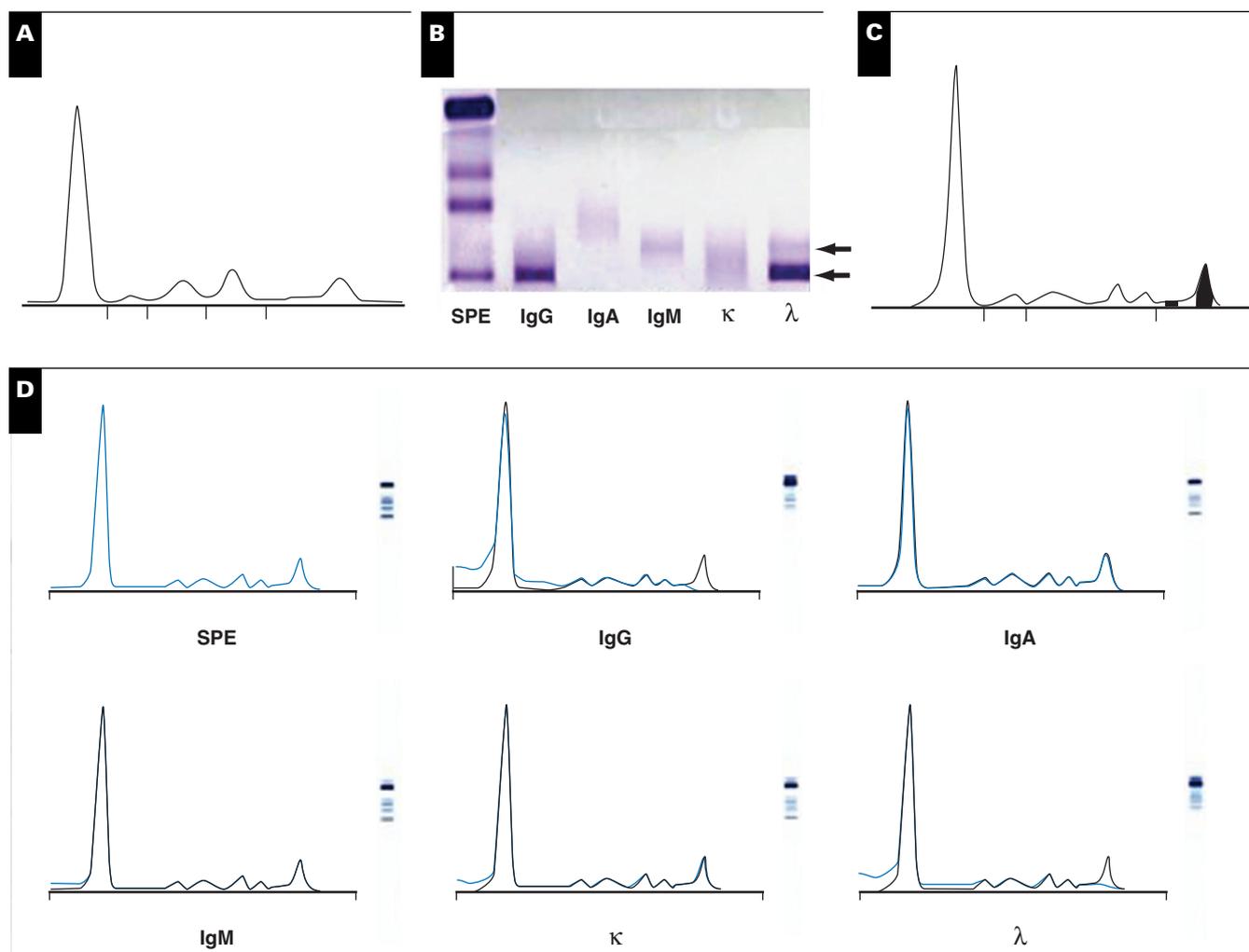


Figure 1 A representative IgM case missed by capillary zone electrophoresis (CZE)/immunofixation. **A**, Agarose gel electrophoresis (AGE). **B**, Immunofixation electrophoresis (IFE). Arrows indicate monoclonal bands. **C**, CZE. **D**, Immunofixation. Monoclonal bands were identified with AGE and CZE. In addition to an IgGλ band identified by IFE and immunofixation, an IgMλ was also identified by IFE. SPE, serum protein electrophoresis.

cancer. Whether these are the results of clonal progression or an oligoclonal inflammatory response is unknown.

Monoclonal Bands in Specimens Negative by AGE or CZE

In cases that were negative for a monoclonal band based on AGE or CZE, a minority showed monoclonal bands on IFE and/or CZE/immunofixation, although in routine practice, IFE is not usually ordered if no monoclonal band is detected by AGE. For AGE, 5 (8%) of 59 no-band cases had a positive band on IFE; 3 of them were IgA, and only 1 was identified by CZE. The other 2 were free light chains; 1 was identified by CZE but considered normal by CZE/immunofixation.

For CZE, 9 (15%) of 62 no-band cases had positive immunotyping by IFE, and only 2 (3%) of 62 no-band cases had positive immunotyping by CZE/immunofixation. Both were detected by AGE and identified as IgA by IFE, whereas

only 4 of 8 reviewers identified them as IgA by CZE/immunofixation. IgA bands sometimes overlap with the β band, and this may be the reason it is missed on AGE and CZE. Among the other 7 no-band cases by CZE that had bands by IFE, only 1 was diagnosed as multiple myeloma. Four cases had clinical diagnoses normally accompanied by oligoclonal or polyclonal bands, so the clinical significance of the monoclonal IgA in these cases is unknown.

Inconclusive Cases

The inconclusive cases are listed in **Table 5**. Only 1 case was detected by IFE and CZE/immunofixation, but the methods differed on the immunotype. Again, CZE/immunofixation did not detect IgM. One case was diagnosed as renal failure, 2 as liver cirrhosis, and 1 as MGUS. No cases were diagnosed as multiple myeloma.

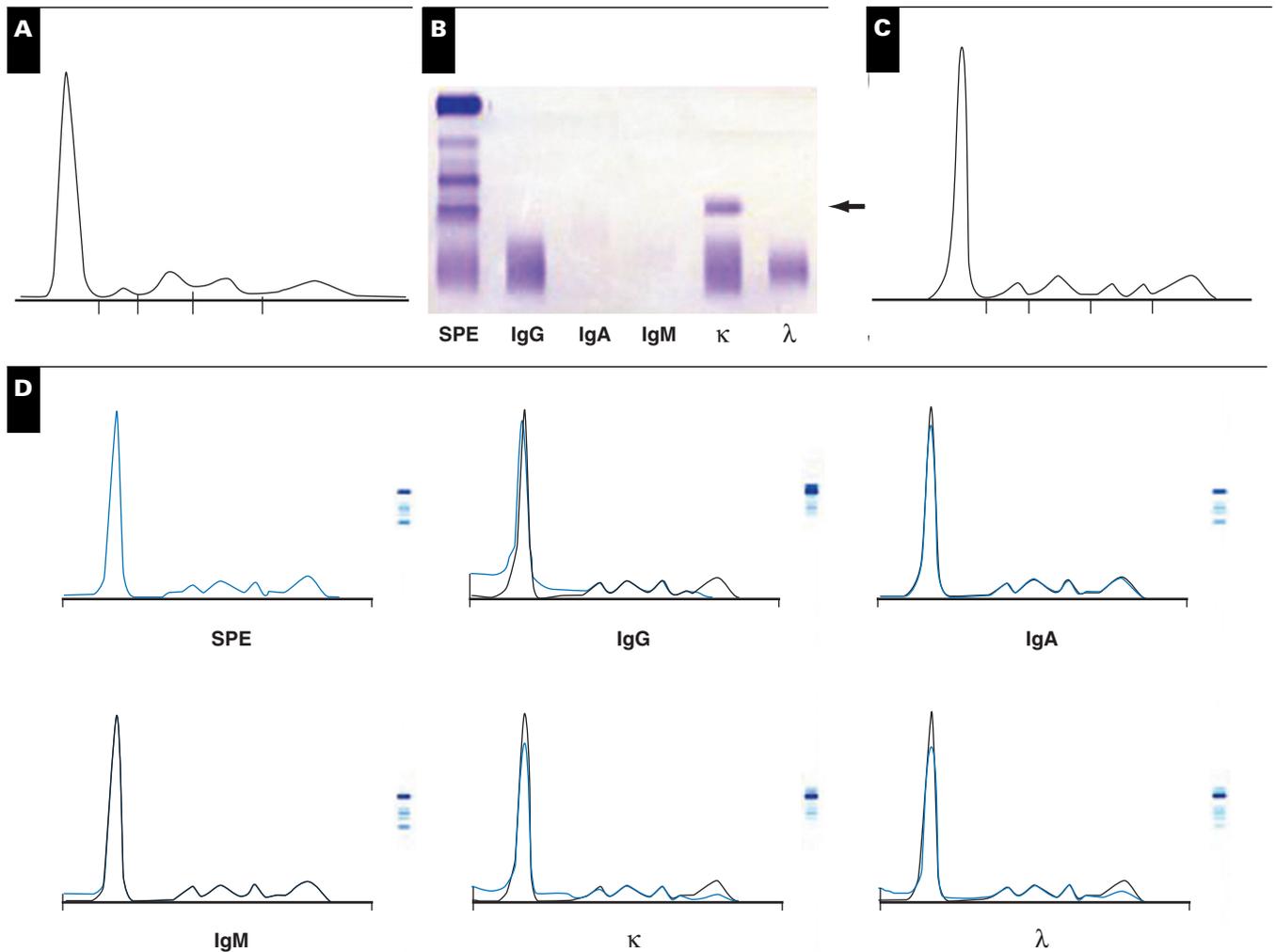


Figure 2 A representative free light chain case missed by capillary zone electrophoresis (CZE)/immunosubtraction. **A**, Agarose gel electrophoresis (AGE). **B**, Immunofixation electrophoresis (IFE). The arrow indicates a monoclonal band. **C**, CZE. **D**, Immunosubtraction. A monoclonal free κ chain was identified with AGE/IFE. No band was identified on CZE. Although reduction of the peak was seen with κ and λ on immunosubtraction, it was interpreted as polyclonal with the expected κ/λ ratio. SPE, serum protein electrophoresis.

Discussion

Automated capillary electrophoresis systems for the analysis of monoclonal paraproteins offer the potential to improve turnaround time and provide cost savings. This is the

first report of the Sebia CAPILLARYS 2 immunotyping method. In addition, this is the first such report to use 8 reviewers to minimize reviewer bias.

The performance of the 2 commercially available capillary systems (Beckman Coulter Paragon 2000 and Sebia

Table 5
Inconclusive Cases by AGE or CZE in Specimens Tested for Monoclonal Proteins

Case No.	AGE	IFE	CZE	Immunosubtraction	Diagnosis
8	No band	Normal	Inconclusive	Normal	Renal failure
9	Inconclusive	Inconclusive	No band	Normal	MGUS
10	No band	IgA*	Inconclusive	Normal	Cirrhosis
11	Inconclusive	IgM λ	Inconclusive	IgG κ	Cirrhosis

AGE, agarose gel electrophoresis; CZE, capillary zone electrophoresis; IFE, immunofixation electrophoresis; MGUS, monoclonal gammopathy of undetermined significance.
 * Identification of light chain inconclusive.

CAPILLARYS) has been evaluated and reviewed,³ and, generally, AGE and CZE showed similar serum protein patterns in various disease entities with an overall concordance of 96% between AGE and CZE.¹¹ Our results demonstrated that the CAPILLARYS 2 CZE has a lesser but similar concordance, 91%, with AGE. By using IFE as the gold standard we found that AGE and CZE had similar performance with respect to paraprotein detection. The sensitivity of CZE was 81% (95% confidence interval [CI], 70%-92%) vs 90% (95% CI, 81%-98%) for AGE, and the specificity for both was 100%, with an overall concordance with AGE of 91%. The measure of agreement (κ) with IFE for the AGE method was 0.90 (95% CI, 0.82-0.99) and for CZE was 0.82 (95% CI, 0.71-0.93). Thus, there was greater accuracy with the AGE method compared with the CZE method. The positive predictive value of both tests was 100%, and the negative predictive values for AGE and CZE were 92% (95% CI, 84%-99%) and 85% (95% CI, 77%-94%), respectively. Once again, the predictive value for AGE was higher than that for CZE; however, this difference was not statistically significant.

AGE and CZE missed some IgA paraproteins. Similar studies have found that CZE may have increased sensitivity and decreased specificity for identifying paraproteins compared with AGE.⁷⁻⁹ Our experience was that CZE did not have increased sensitivity compared with AGE and had the same specificity. The reasons for these differences are unclear; however, there were some differences in the referenced studies compared with our study. In the study by Litwin et al,⁹ the AGE method was different from ours, raising the possibility that the Sebia AGE is more sensitive than the method they used. Bossuyt et al⁷ found that the CAPILLARYS CZE system had a lower specificity than the AGE and the Beckman Paragon 2000 CZE. Differences in this study include the fact that double the number of samples were evaluated compared with our study, and it is unclear who or how many people identified the bands in AGE/CZE. Lissioir et al⁸ noted that the CAPILLARYS CZE displayed a higher sensitivity (97.2%) and lower specificity (93.7%) than the Sebia AGE (93.5% and 98.9%, respectively). It is possible that we had more cases of IgM and light chain monoclonal gammopathies than did Lissioir et al,⁸ who did not elaborate on the identity of the monoclonal gammopathies.

The percentage of agreement on the immunotype between immunosubtraction and IFE was 86%. Immunosubtraction did not identify some IgM and free light chain paraproteins, especially when they were at a low concentration. This may indicate that IFE is more sensitive than immunosubtraction for the detection of those paraproteins.

Potential shortcomings of the present study include limited training and interpretation experience with the CAPILLARYS 2 CZE/immunosubtraction system. Of note, Sebia has recently increased its training for this system. Also,

the numbers of samples are limited. Nevertheless, we showed that CZE/immunosubtraction was comparable to IFE in the detection of other immunotypes, including IgG, IgA (both methods missed some cases), and bound light chains. However, CZE/immunosubtraction did not detect all 6 IgM cases and some free light chains in the initial collection of samples. Suboptimal performance of CZE in the detection of IgM, IgA, and free light chains has been reported.¹²⁻¹⁶ The reason for this is unclear, but it could be related to the particular migration patterns of these paraproteins. This phenomenon has been noted in CZE/immunosubtraction in the detection of some IgA paraproteins (which often migrate in the β region) and other paraproteins in the slow γ region.¹⁷

Concerning the apparent serum free light chains missed with CZE/immunosubtraction, light chains are most often detected in urine, and the current version of the CAPILLARYS 2 applies only to serum samples. Thus, the important comparison of urine IFE and immunosubtraction has not been made for the Sebia system. The monoclonal proteins missed by CZE were most likely at low concentrations. It should be noted that Litwin et al⁹ showed that CZE/immunosubtraction is superior to AGE in the identification of all paraproteins, including IgM and IgA; however, their study was with a different CZE system. In our collection of additional samples, all 5 IgM paraproteins were correctly identified by CZE/immunosubtraction. The lowest concentration in these samples was 545 mg/dL (5.45 g/L), which is much higher than the reported detection limit of 75 mg/dL (0.75 g/L) for IgM in the literature¹¹ or 25 mg/dL (0.25 g/L) as stated by Sebia (package insert). Of interest, another sample that measured 500 mg/dL (5.00 g/L) of IgM by nephelometry was very difficult to identify by CZE/immunosubtraction (data not shown), raising the possibility that all IgM monoclonal proteins are not being recognized solely on the basis of concentration. Although we did not determine the concentration of the first 6 IgM paraproteins, the bands were relatively weak on IFE (Figure 1). Two modifications have been proposed to improve the ability of CZE/immunosubtraction to detect low concentrations of IgM, namely the use of 2-mercaptoethanol, to get monoclonal IgM into monomeric form,¹⁸ and changing of the ionic strength and pH of the buffer.⁵ Whether these modifications will improve the detection of IgM in this CZE/immunosubtraction system remains to be determined.

MGUS is considered a high-risk, premalignant condition. Based on a series of 1,384 patients studied at the Mayo Clinic, Rochester, MN, the risk of progression was 1% per year, even after more than 25 years of stable paraprotein concentrations. It was shown that the presence of IgM or IgA paraprotein and an abnormal serum free light-chain ratio were risk factors for progression.¹⁹ IgM-related disorders include Waldenström macroglobulinemia and IgM multiple myeloma. The former disease often manifests with hyperviscosity syndrome, which

requires emergency intervention to prevent devastating consequences. Although IgM myeloma is rare (<1%), it may have some clinical features that overlap with Waldenström macroglobulinemia.²⁰ In addition, up to 20% of myeloma cases have no detectable heavy chain in the serum. Thus, it is imperative to correctly determine IgM and free light chain paraproteins to diagnose the disease and monitor its progression. In this regard, the performance of the CAPILLARYS 2 CZE/immun subtraction is less sensitive than IFE, especially for the detection of low concentrations of paraproteins, which may signal an early stage of the disease. In fact, a recently proposed diagnostic criterion for Waldenström macroglobulinemia includes IgM monoclonal gammopathy of any concentration, among other features.²¹

The CAPILLARYS 2 offers increased sample throughput for serum protein electrophoresis. In our hands, a typical 30-well AGE takes approximately 1.5 hours, whereas the CZE offers 90 samples per hour. A 4-sample IFE gel also takes about 1.5 hours, while CZE/immun subtraction does 10 samples per hour. On the other hand, the reagent costs are more for the CZE/immun subtraction. Overall, the costs of running either system are similar in our hands. Serum IgD and IgE, urine electrophoresis, and cerebrospinal fluid electrophoresis are not offered on the CZE/immun subtraction system at this time. Having both systems provides significant backup capability for serum protein electrophoresis.

Based on our experience, the performance of CAPILLARYS 2 CZE/immun subtraction is similar to that of AGE/IFE in terms of specificity; however, the detection of monoclonal paraproteins and their immunotyping seems less sensitive for IgM and, possibly, for free light chains.

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