

Comparison of Capillary Zone and Immunosubtraction With Agarose Gel and Immunofixation Electrophoresis for Detecting and Identifying Monoclonal Gammopathies

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Abstract

Capillary zone electrophoresis (CZE) and immunosubtraction electrophoresis (ISE) were evaluated for ability to detect and immunotype monoclonal proteins, compared with agarose gel electrophoresis (AGE) and immunofixation electrophoresis (IFE), respectively. Six hundred seventeen serum samples were analyzed with CZE and AGE to determine sensitivity and specificity in detecting IFE-confirmed monoclonal gammopathies. Both techniques detected all monoclonal spikes due to IgM ($n = 8$), IgG ($n = 38$), and free light chains ($n = 3$). Agarose gel electrophoresis, however, detected only 11 of 14 (79%) IgA monoclonal spikes detected with CZE. In a second study, 78 serum samples, 48 of which had a monoclonal gammopathy confirmed with IFE, were evaluated with ISE. Only 60% to 75% of the monoclonal gammopathies were correctly immunotyped with ISE by 4 readers blinded to the IFE immunotype. Thus CZE was more sensitive than AGE in detecting low concentrations of monoclonal proteins, but ISE is less accurate than IFE in determining the immunotype of the monoclonal gammopathy.

Serum protein agarose gel and immunofixation electrophoresis are valuable tools for detection and identification of monoclonal gammopathies, amyloidosis, and other dysproteinemias. Monoclonal gammopathies are found in 3% of patients older than 70 years and 1% of patients older than 50.^{1,2} Initially, small amounts of monoclonal proteins produce no clinical symptoms, and the diagnosis is monoclonal gammopathy of unknown significance.³ Inasmuch as 20% to 25% of patients may eventually develop the malignant disorder multiple myeloma, it is important that sensitive methods be available to detect these monoclonal proteins. For more than 20 years, monoclonal proteins in serum and urine have been detected primarily with agarose gel electrophoresis (AGE). Capillary zone electrophoresis (CZE) is a rapid, cost-effective, partially automated alternative to AGE.⁴⁻¹⁰ This method involves separation of charged molecules in a buffer-filled capillary tube by application of high voltage (30 kV).¹¹ The main beneficial characteristic of CZE is the high separation efficiency that can be achieved, which allows the assay to be completed in approximately 1 minute. Capillary zone electrophoresis has been reported to have increased sensitivity and comparable specificity to AGE in identification of monoclonal proteins.⁴⁻¹⁰

Immunotyping of paraproteins after detection on agarose gel is usually performed with immunofixation electrophoresis (IFE).^{12,13} Recently an alternative method to immunofixation has been developed for immunotyping monoclonal gammopathies by subtraction with immunoabsorption and subsequent electrophoresis with CZE. With immunosubtraction electrophoresis (ISE), the serum is first incubated with immunoglobulin class-specific antibodies bound to Sepharose beads. The serum from which 1 immunoglobulin class or light chain has been "subtracted" with the antibody-coated Sepharose beads is then run on the CZE instrument, and the

electrophoretic pattern is examined for decrease in or disappearance of the monoclonal protein.¹⁴

We used an automated serum protein electrophoresis system to examine the feasibility of utilizing CZE and ISE to identify and immunotype monoclonal serum proteins.

Methods

Agarose Gel Electrophoresis

Agarose gel electrophoresis was performed with the REP system (Helena Laboratories, Beaumont, TX). Gels were stained with Ponceau S and scanned with a densitometer. Densitometer readings were relayed to the central processing unit (Compaq Deskpro 286, Intel 80286), which calculated the area of each peak and expressed it as a percentage of the total protein.

Capillary Zone Electrophoresis

Capillary zone electrophoresis was performed with a Paragon CZE 2000 system (Beckman Instruments, Brea, CA), using the automated sample-handling module, Beckman system buffers, and Beckman software designed for the 7-capillary clinical instrument.

Immunologic Techniques

The procedure for immunofixation was followed according to established protocols.¹⁵ Immunotyping by immunosubtraction was performed with the Beckman Paragon CZE 2000 after incubation of serum aliquots with γ , α , μ , κ , and λ light chain antibodies bound to Sepharose beads. After incubation of the

serum aliquots with antibody-coated beads, CZE was performed to determine which reagents removed the monoclonal proteins. The electropherograms from the original and absorbed serum aliquots were analyzed with Beckman Paragon CZE 2000 software. Quantitation of specific immunoglobulins was accomplished with nephelometry using manufacturer-recommended reagents on a Beckman array.

Clinical Samples

The correlation study of AGE and CZE was conducted by testing 617 patient serum samples with each method. Patterns of CZE and AGE on random samples were interpreted by a medical technologist (S.K.A. or G.P.) and a medical director (C.M.L. or H.R.H.) blinded to IFE immunotype. Reference ranges for CZE used for correlation were Beckman published ranges for a population of 134 healthy men and women in southern California.

For evaluation of sensitivity and specificity of the ISE method, 78 patient sera were tested, 48 of which were determined to have a monoclonal protein as determined with IFE. The interpretations of the ISE patterns were made by the same 2 medical technologists and 2 medical directors, blinded to the IFE results.

Results

When compared with AGE, the increased resolution of CZE allows separation of the β region into 2 distinct peaks, transferrin and the third component of complement (C3) (Figure 1). There was 100% agreement between CZE and

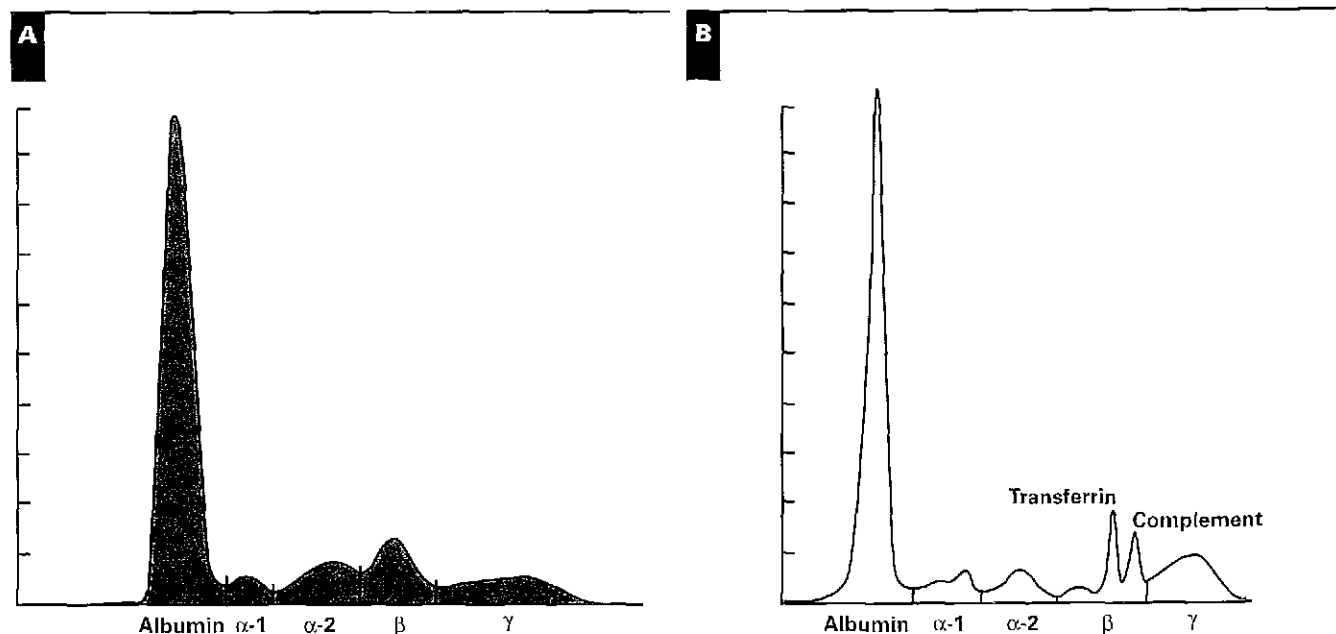


Figure 1 Electrophoresis of normal serum proteins. A, Agarose gel electrophoresis. B, Capillary zone electrophoresis.

AGE on 552 serum samples that did not contain a monoclonal protein (Table 1). Neither the presence of fibrinogen nor hemoglobin (hemolyzed samples) caused artifacts on the CZE electropherogram, which are noted as an extra peak in the β region or a suspect band on AGE, respectively. Each method detected a monoclonal spike in 8 serum samples with IgM monoclonal gammopathy and in 38 serum samples with IgG monoclonal gammopathy. Both methods also detected 3 free λ light chain and 2 biclonal gammopathies. In

Table 1
Capillary Zone Electrophoresis vs Agarose Gel Electrophoresis in Detection of Monoclonal Proteins

Monoclonal Gammopathy	No. of Serum Samples	Gammopathy Detected	
		CZE (%)	AGE (%)
No monoclonal protein detected	552	0	0
IgM	8	100	100
IgG	38	100	100
IgA	14	100	79
Free λ light chain	3	100	100
Biclonal	2	100	100
Total	65	100	95

CZE = capillary zone electrophoresis; AGE = agarose gel electrophoresis.

contrast, AGE did not detect 3 of the 14 IgA monoclonal gammopathies detected with CZE (79%). The AGE, CZE, and IFE profiles of serum from a patient with an IgA- λ monoclonal gammopathy are shown in Figure 2. Agarose gel electrophoresis showed a normal pattern (Figure 2A), but CZE demonstrated a peak within the β region next to the transferrin peak (Figure 2B). The sample was tested with IFE, which confirmed the presence of IgA- λ monoclonal gammopathy (Figure 2C). The 3 IgA monoclonal proteins not detected with AGE were tested with nephelometry to quantitate the amount of IgA present. These paraproteins demonstrated total IgA values less than 650 mg/dL. All of the discrepant IgA monoclonal gammopathies were "hiding" in the β region on the agarose gel, as revealed with IFE.

Because CZE separates the transferrin and C3 proteins into distinct peaks, we had difficulty determining whether increases in transferrin or complement peak height might be hiding a small monoclonal protein. With AGE, a suspicious band of restriction was noted in the β region (Figure 2D), and with CZE increased complement and normal transferrin were demonstrated (Figure 2E). IgA- λ monoclonal gammopathy was confirmed in the β area with IFE (Figure 2F).

Seventy-eight serum samples were examined to evaluate the feasibility of using ISE to immunotype monoclonal

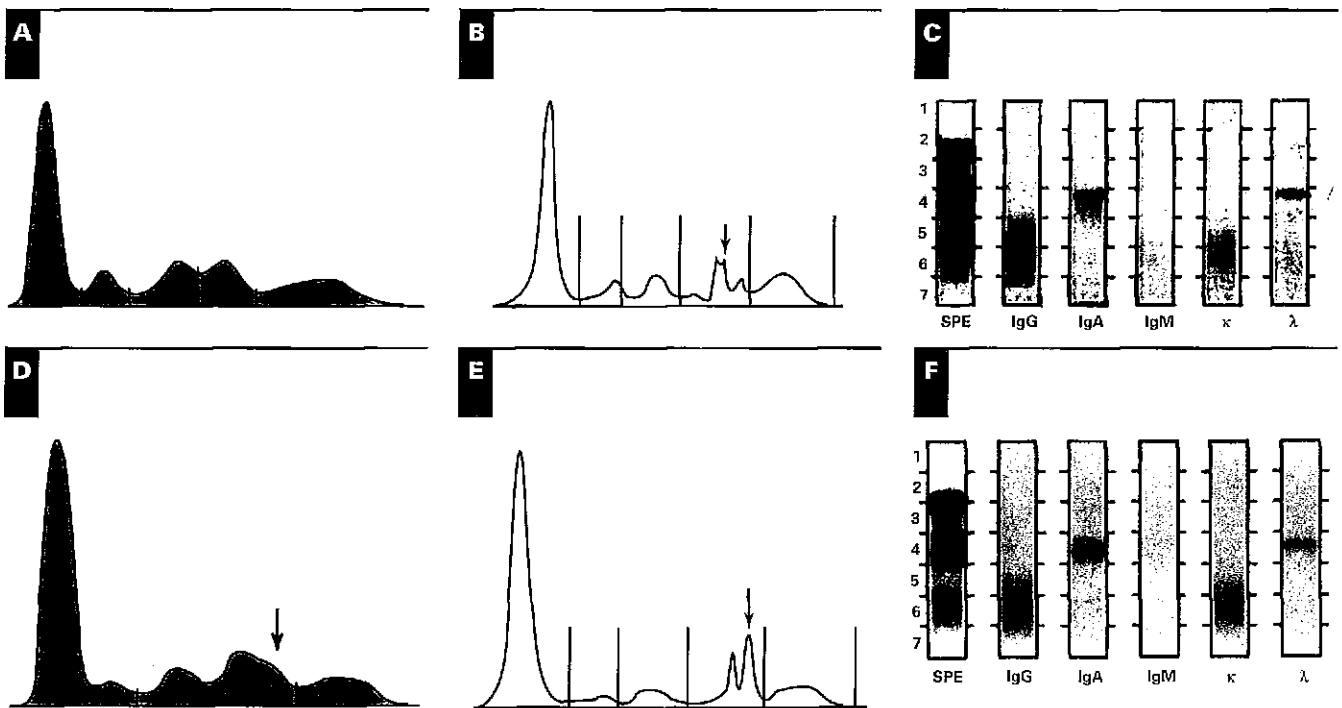


Figure 2 Electropherograms of serum samples from 2 patients with IgA- λ monoclonal gammopathy. A, Agarose gel electrophoresis (AGE) tracing shows a normal tracing in this patient. B, Capillary zone electrophoresis (CZE) tracing of same serum as in A shows a band in the β region. Arrow indicates monoclonal protein. C, Immunofixation electrophoresis (IFE) tracing shows IgA- λ specificity of monoclonal protein. D, AGE tracing in second patient shows a suspect area in β region (arrow). E, CZE tracing shows increased complement peak (arrow). F, IFE tracing shows IgA- λ monoclonal gammopathy.

gammopathics. Forty-eight of the samples contained monoclonal proteins. The samples were originally immunotyped with IFE. The ISE patterns were read by 4 readers (2 medical technologists, 2 medical directors) blinded to the IFE immunotype. The IFE immunotypes of the 48 samples containing monoclonal proteins were identified as follows: 12 were IgG- κ , 10 IgG- λ , 10 IgM- κ , 7 IgA- κ , 3 IgA- λ , 3 free κ , 2 free λ , and 1 biclonal IgM- λ and IgM- κ . The remaining 30 samples were either normal, polyclonal, or had a decreased amount of gammaglobulins.

With ISE, 2 readers correctly identified all 30 negative control samples as negative for a paraprotein (Table 2); the other 2 readers, however, incorrectly identified a paraprotein peak in 3% and 7%, respectively, of the negative control samples. The most common immunotype given for these negative control samples was IgG gammopathy (2 of 3). Accuracy for correct immunotyping of the monoclonal gammopathies with ISE by the 4 independent readers was 64% to 91% for IgG gammopathies, 30% to 40% for IgM gammopathies, 90% to 100% for IgA gammopathies, 40% to 80% for free light chain gammopathies; none of the reviewers correctly identified the biclonal IgM- κ and IgM- λ . Overall, the accuracy of the 4 readers in correctly identifying and immunotyping monoclonal proteins was between 60% and 75%. In the majority of missed ISE monoclonal samples, the incorrect answer was given as a polyclonal or normal pattern. In general, serum samples containing missed monoclonal gammopathies had small quantities of a monoclonal protein or the monoclonal spike was present in the background of a polyclonal increase in immunoglobulins. In these cases, there were subtle differences on the electropherogram after subtraction with the immunoglobulin or free light chain-specific antibody-coated beads. Large monoclonal spikes, however, were correctly identified by all 4 readers with ISE.

An example of an ISE pattern identified correctly by all 4 readers is illustrated in Figure 3. A large monoclonal spike is demonstrated in the γ region (Figure 3A), which at IFE is identified as an IgA- κ monoclonal gammopathy with

dimerization (Figure 3B). Compare Figure 3C with Figure 3D and 3E, which shows subtraction of the monoclonal protein with the IgA- and κ -specific reagents, respectively.

An example of an ISE pattern in which all readers incorrectly immunotyped the monoclonal protein is shown in Figure 4, which illustrates the difficulty in interpreting an ISE pattern when relatively low amounts of the monoclonal protein are contained in a background of a polyclonal increase in total immunoglobulins. Figure 4A shows a monoclonal spike in the γ region, which was identified as an IgM- κ gammopathy at IFE (Figure 4B). All 4 readers incorrectly interpreted the pattern as free κ , IgG- κ polyclonal, and polyclonal. Only subtle differences are noted in the sharpness and height of the peak in the γ region in Figure 4C-4E and 4G, compared with the electropherogram in Figure 4A.

Discussion

The CZE system, in comparison with AGE, demonstrated increased sensitivity for small monoclonal gammopathies, especially those of the IgA isotype. The agarose gel method used was not a high-resolution agarose gel, which may have contributed to the discrepancy between the systems. A similar study by Jenkins et al⁴ compared CZE and high-resolution AGE. These authors also demonstrated IgA monoclonal proteins hiding in the β region, which were later identified with CZE. Similar findings with IgA monoclonal gammopathies were described in a study by Henskins et al.⁶ In a study by Katzmann et al,¹⁶ of 215 serum samples in which a monoclonal protein was detected, 4 monoclonal light chain and 3 IgA samples appeared normal with AGE but abnormal with CZE. In addition, there were 5 monoclonal light chain and 3 monoclonal IgA samples in which no abnormality was detected with AGE or CZE. Jolliff and Blessum⁹ also described improved ability to detect IgA and IgM monoclonal gammopathies with CZE. As a note of

Table 2
Monoclonal Gammopathies Detected With Immunofixation Electrophoresis

Detected With IFE	No. of Serum Samples	Reviewer			
		1	2	3	4
No monoclonal protein detected	30	29 (97%)	30 (100%)	30 (100%)	28 (93%)
Monoclonal protein detected	48	35 (73%)	29 (60%)	31 (64%)	36 (75%)
IgG	22	17 (77%)	14 (64%)	16 (73%)	20 (91%)
IgM	10	4 (40%)	4 (40%)	3 (30%)	4 (40%)
IgA	10	10 (100%)	9 (90%)	9 (90%)	10 (100%)
Free light chains	5	4 (80%)	2 (40%)	3 (60%)	2 (40%)
Biclonal IgM- κ , IgM- λ	1	0 (0%)	0 (0%)	0 (0%)	0 (0%)

IFE = immunofixation electrophoresis.

*Percentage includes only those with correct immunophenotype; κ and λ gammopathies grouped together.

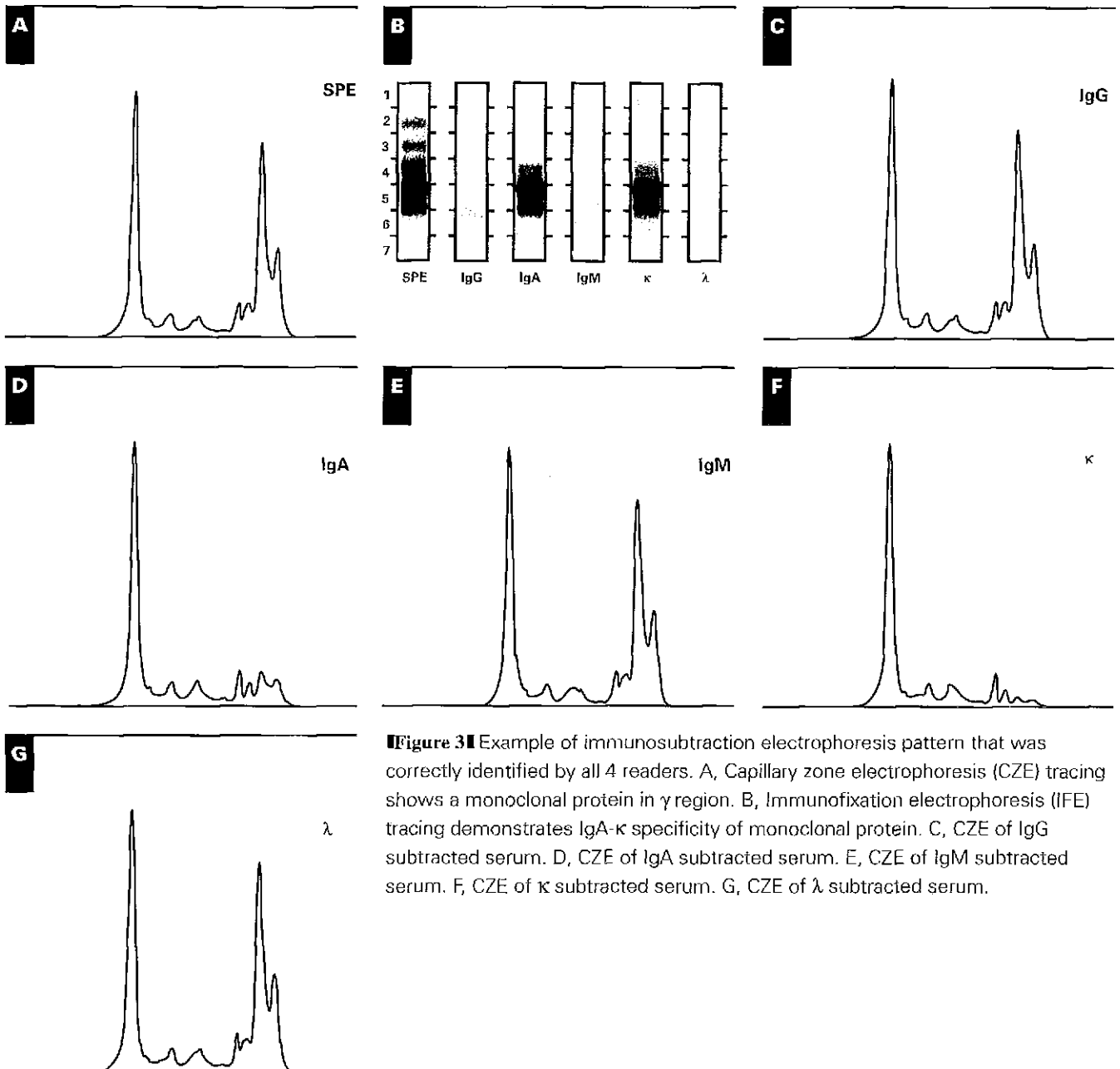


Figure 3 Example of immunofixation electrophoresis pattern that was correctly identified by all 4 readers. A, Capillary zone electrophoresis (CZE) tracing shows a monoclonal protein in γ region. B, Immunofixation electrophoresis (IFE) tracing demonstrates IgA- κ specificity of monoclonal protein. C, CZE of IgG subtracted serum. D, CZE of IgA subtracted serum. E, CZE of IgM subtracted serum. F, CZE of κ subtracted serum. G, CZE of λ subtracted serum.

caution, IgA monoclonal proteins can also hide within the transferrin or complement peak on CZE (see Figure 2). Therefore, care must be taken in evaluating the possibility of monoclonal proteins hiding within these peaks in the β region on CZE. Abnormal increases in either the transferrin or complement peak should be treated as suspicious for the presence of a monoclonal protein and evaluated with an immunotyping procedure.

Another advantage of CZE over AGE is the absence of application point artifact, observed in previous studies.¹⁷ Application point artifact is not seen with CZE because the sample is injected into the inlet of the capillary. Suspect tracings in the γ

region caused by hemolysis of the serum specimen were also eliminated with CZE. When plasma is analyzed with AGE, a fibrinogen band is usually noted in the β region. We did not detect a fibrinogen band on plasma samples tested with CZE. Similar observations have been made by others.^{7,8} A disadvantage of CZE, however, is that it has not yet been adapted for electrophoresis of urine.

Capillary zone electrophoresis is an automated system for serum protein electrophoresis that allows processing of 42 samples in 1 hour. Once the CZE system is running, it does not require intervention ("walk away" instrument). The AGE system, however, requires a technician to be present for timed

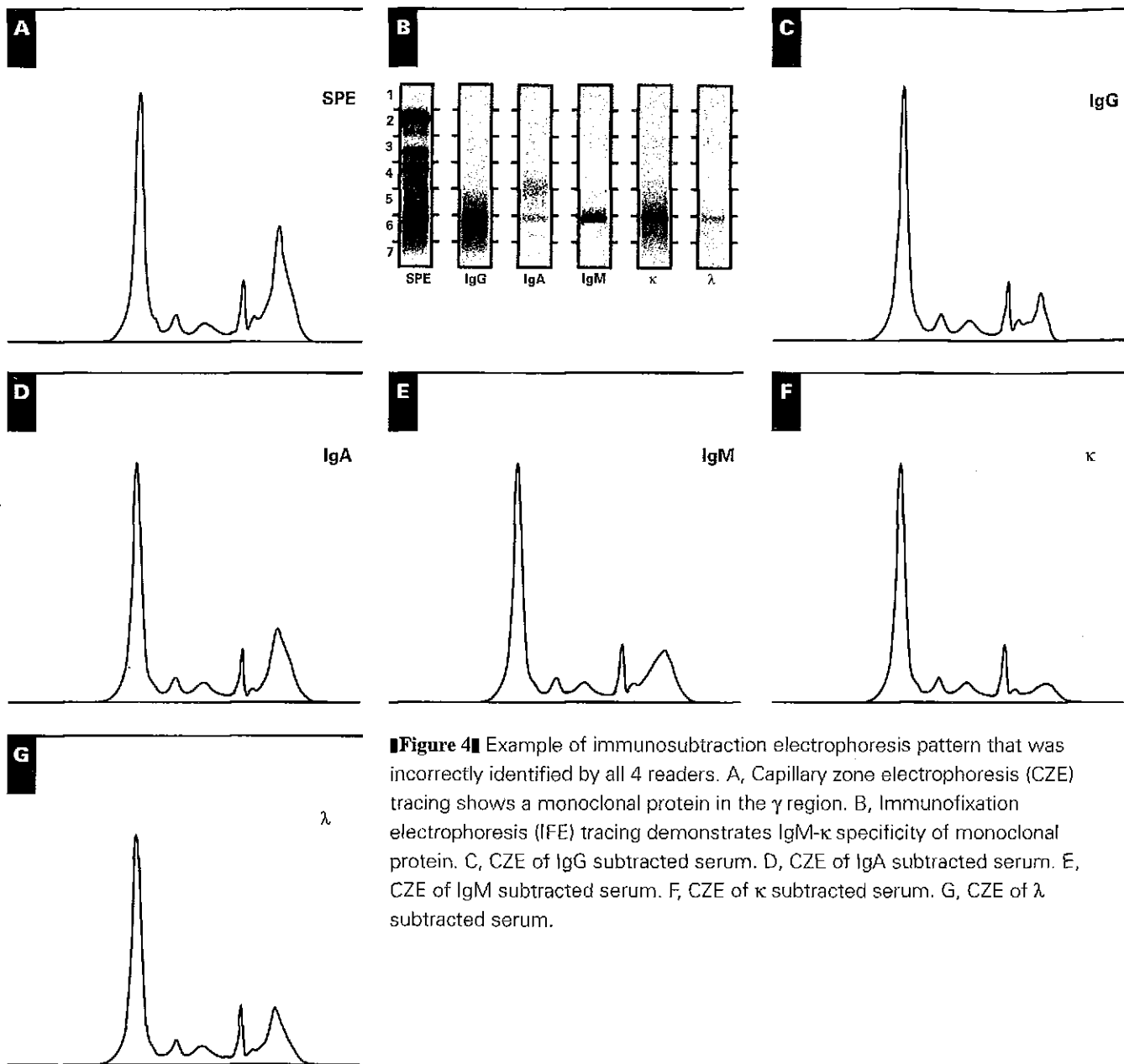


Figure 4 Example of immunosubtraction electrophoresis pattern that was incorrectly identified by all 4 readers. A, Capillary zone electrophoresis (CZE) tracing shows a monoclonal protein in the γ region. B, Immunofixation electrophoresis (IFE) tracing demonstrates IgM- κ specificity of monoclonal protein. C, CZE of IgG subtracted serum. D, CZE of IgA subtracted serum. E, CZE of IgM subtracted serum. F, CZE of κ subtracted serum. G, CZE of λ subtracted serum.

steps such as electrophoresis completion and staining. Labor determined from time measurements of a CZE instrument run compared with AGE reflected a 49% saving in time.

When a monoclonal protein is suspected on serum protein electrophoresis, it must be characterized by immunotyping to confirm the diagnosis of monoclonal gammopathy. Because IFE is more labor intensive than AGE, it was hoped that ISE with CZE would be a cost-effective alternative. We found, however, that the ISE patterns were difficult to interpret and fraught with uncertainty. Suspect peaks identified on the routine CZE electropherogram could not be immunotyped with ISE with the same level of confidence as with IFE. Conversely, normal-appearing CZE electropherograms

with abnormal appearing ISE patterns occasionally confused 2 readers; this was reflected in false-positive identification of a monoclonal protein in 3% and 7% of negative controls. Polyclonal patterns were often misinterpreted as IgG monoclonal proteins, because immunoabsorption removed most of the increase in immunoglobulins. All readers scored particularly poorly in ability to correctly immunotype monoclonal proteins. Identification of the IgM isotype was most inaccurate, with only 30% to 40% correctly identified. Complicated patterns such as biconal IgM- κ and IgM- λ were difficult to interpret correctly; no reader was able to correctly identify the ISE pattern. Incorrectly interpreted ISE patterns tended to occur with small monoclonal proteins contained in the

background of a polyclonal increase, where subtraction of the monoclonal protein was sometimes difficult to see.

In contrast to our study, Katzmann et al¹⁶ reported comparable sensitivity with ISE and IFE. They examined 22 samples in which there were discordant results in detection of a monoclonal protein, where IFE findings were negative but AGE or CZE detected a monoclonal band. With ISE, 10 of these samples were identified as containing a small monoclonal protein. Conversely, 22 samples were positive for monoclonal gammopathy with IFE and negative for a monoclonal spike with either AGE or CZE. Seven of these samples were negative with ISE.

Katzmann et al¹⁶ did, however, reach some of the same conclusions with ISE as in our study. They reported problems with immunotyping light chain and biclonal gammopathies with ISE. They found that ISE missed 1 of the monoclonal gammopathies in 3 of 8 biclonal gammopathies. They also believe that IFE may still be needed as a complementary method for detection of free light chains, detection of a second small monoclonal gammopathy in a sample with biclonal gammopathy, and in hypogammaglobulinemia samples with no M spike. They also found that a small monoclonal protein within a polyclonal increase often required multiple dilutions for interpretation with ISE.

It is possible that with experience a higher level of accuracy can be attained, allowing detection of small amounts of monoclonal proteins with ISE, once the reader becomes accustomed to the absence of a suspect monoclonal peak after subtraction. Nevertheless, identifying the presence of a monoclonal band on IFE gel is much easier than trying to detect the absence of a peak with ISE, and will probably remain a much easier skill to master.

The overall accuracy of only 60% to 75% with ISE in immunotyping monoclonal proteins in our study is unacceptable. Discrete and prominent monoclonal peaks, however, were interpreted without difficulty by all readers with ISE. Therefore, an alternative use of the ISE technique might be to immunotype only discrete and prominent monoclonal proteins that are readily identified on CZE. Any ISE pattern that does not appear to be definitive should then be further evaluated with IFE. Capillary zone electropherograms with suspicious, small abnormal bands would automatically be analyzed for monoclonal proteins with IFE. The cost-effectiveness of this screening technique needs further evaluation. Clearly, CZE offers significant advantages over AGE in sensitivity and labor savings. In contrast, at least at present, ISE cannot be recommended for routine immunotyping of monoclonal proteins.

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