Silver Staining of Unconcentrated Cerebrospinal Fluid in Agarose Gel (Panagel) Electrophoresis

Pankaj D. Mehta, Sangita P. Mehta, and Bruce A. Patrick

We subjected cerebrospinal fluid (CSF) from 20 patients with multiple sclerosis and 20 patients with other neurological diseases to agarose gel (Panagel) electrophoresis followed by staining with silver. Ten microliters of unconcentrated CSF from multiple sclerosis patients containing 0.4 to 0.8 μg of immunoglobulin G was found to be optimum for detection of oligoclonal IgG bands, so identified by immunofixation. The band patterns for unconcentrated CSF stained with silver were almost identical to those for the same CSF concentrated 40-fold and stained with Coomassie Brilliant Blue. Silver staining thus enables the clinical laboratory to electrophorese unconcentrated CSF on commercially prepared (Panagel) plates.

Additional Keyphrases: multiple sclerosis, neurological disorder, oligoclonal IgG

Demonstration of oligoclonal IgG in CSF assists in the diagnosis of MS (1–3). Commercially available agarose-gel plates, reagents, and electrophoresis unit (Panagel electrophoresis system; Worthington Diagnostics, Freehold, NJ) are commonly used in such demonstrations. A disadvantage of the system is that the CSF must be concentrated about 40- to 60-fold before being applied to the gel because the stain that is commonly used, Coomassie Brilliant Blue, is too insensitive to reveal oligoclonal bands in the unconcentrated fluid. The present study was undertaken to see if unconcentrated CSF run in “Panagel” plates can be stained with silver and to compare the band patterns for unconcentrated CSF with those for concentrated CSF.

Materials and Methods

Collection of CSF. We collected 3 mL of CSF from 20 patients with clinically definite MS and 20 with other neurological diseases. The other neurological diseases include central nervous system lupus, acute idiopathic polyneuritis, Guillain–Barré syndrome, seizure, Huntington’s disease, and headache.

Concentration of CSF and IgG determination. The CSF specimens were concentrated 40-fold by ultrafiltration (Model 12 ultrafilter; Amicon Corp., Lexington, MA) or were dialyzed and lyophilized. The IgG in unconcentrated and concentrated CSF was quantified by radial immunodiffusion (L.C. Partigen Plate; Calbiochem-Behring, La Jolla, CA 92037) with the use of goat antiserum specific to human IgG.

Panagel electrophoresis and silver staining. This system consists of agarose gel bonded to a plastic film. We applied to the gel 10 μL of unconcentrated CSF containing 0.4 to 0.8 μg of IgG and electrophoresed according to the manufacturer’s instructions as previously described (4).

We silver-stained the gel according to Willoughby and Lambert (5) with minor modifications. Briefly, the procedure is as follows.

After electrophoresis, fix the gel for 10 min in 100 mL of an aqueous solution containing, per liter, 35 g of sulfosalicylic acid, 50 g of trichloroacetic acid, and 50 g of zinc sulfate. Wash the gel for 1 h, with agitation, in four changes of water, then soak it in 95% ethanol for 10 min, blot it in Whatman No. 3 filter paper for 15 min, and dry it in a current of warm air. Again wash the gel for 5 min in water and dry.

For silver staining, prepare two solutions: solution A, containing 5 g of Na2CO3 in 100 mL of water, and solution B, containing 0.4 g of ammonium nitrate, 0.4 g of silver nitrate (Accurate Chemical Corp., Hicksville, NY), 1.0 g of silicotungstic acid (Platz & Bauer, Inc., Stamford, CT), and 2.8 mL of a 370 g/L solution of formaldehyde in 200 mL of water. Add solution B to solution A with constant stirring, in a volume ratio of 2:1, just before staining and pour the mixture into the staining dish containing the dried Panagel. Agitate the dish and when the color of mixture changes from white to gray after about 3 min, remove the gel and immerge it in water. Meanwhile, freshly prepare another mixture of stain and again stain the gel with it. When the gel patterns show the desired intensity (usually after 2 to 3 min), stop the reaction by immersing the gel in a 10 mL/L solution of acetic acid for 15 min, then rinse it in water for 5 min and dry.

Electrophoresis of concentrated CSF. Apply to the gel 10 μL of concentrated CSF containing 10 to 20 μg of IgG and electrophorese in an identical manner (4), except stain the gels with Coomassie Brilliant Blue R (Sigma Chemical Co., St. Louis, MO).

Immunofixation. We electrophoresed 10 μL of unconcentrated CSF from a multiple sclerosis patient, as follows. To the gel surface, apply cellulose acetate strips (Sepharose III; Gelman Instrument Co., Ann Arbor, MI) soaked with three-fold diluted goat antiserum to human IgG (Meloy Laboratories, Springfield, VA). Incubate the gel in a humid chamber for 1 h, wash in 0.15 mol/L NaCl for 24 h, then rinse in water for 30 min and stain with silver as described above.

Results

Figure 1 shows typical Panagel electrophoretic patterns of concurrently run unconcentrated and concentrated CSF.
The albumin band in concentrated CSF is thicker than that seen in unconcentrated CSF, but the γ-globulin region shows no notable differences between concentrated and unconcentrated CSF, either in size or mobility. Band patterns for unconcentrated CSF from 20 MS and the 20 other neurological disease patients, when stained with silver and compared with those for the same CSF run in concentrated form and stained with Coomassie Brilliant Blue, were almost identical.

Figure 2 shows the band patterns for unconcentrated CSF after immunofixation and staining with silver. The oligoclonal bands were identified as IgG.

Discussion

Some investigators (6, 7) have subjected unconcentrated CSF to electrophoresis on agar gel, followed by silver staining, but the method has not been previously applied to Panagel. Although silver staining of proteins after electrophoresis in another commercial agarose gel (Corning Universal System; Corning Glass Works, Medfield, MA) has been described (8), the investigators did not analyze CSF from MS cases. In our experience the staining procedure used by previous investigators (6–8) was unsatisfactory for the Panagel system, because of excessive background staining.

Willoughby and Lambert (5) described silver staining for IgG in agarose gel, but they did not analyze CSF. Here we have shown that their method (5) can be satisfactorily applied to band patterns of unconcentrated CSF with little or no background in a commercially available system. Electrophoresis and staining can be completed in less than 3 h, which is quicker than earlier methods (6–8), and previous reports (6, 7) did not indicate whether or not silver staining can be applied to immunofixed patterns after electrophoresis of unconcentrated CSF. We have shown that, in the Panagel system, specific IgG bands can be identified in the pattern produced with unconcentrated CSF.

Silver staining has recently been used with unconcentrated CSF after isoelectric focusing and immunofixation (9–11). Although isoelectric focusing is more sensitive than Panagel electrophoresis, the latter currently is widely used in clinical laboratories because of its simplicity, rapidity, and low cost. Panagel band patterns of unconcentrated and concentrated CSF were essentially the same in the γ-globulin region, so we conclude that the method is practical for routine use in clinical laboratories in examining unconcentrated CSF for detection of oligoclonal bands.

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References