We describe use of a microconcentrator membrane with a 30,000-Da cutoff for treatment of cerebrospinal fluid (CSF) specimens before detection of oligoclonal bands by electrophoresis on agarose. After centrifugation at 2000 × g for 25 min, 0.5-, 1.0-, and 2.0-ml aliquots of CSF were concentrated 15-, 25-, and 40-fold. Analytical recovery of immunoglobulins G and A from the microconcentrators was about 90% (CV 5–8%). We found good correlation between results by this method and by a silver-stain procedure in a study comparing oligoclonal bands in CSF from multiple sclerosis patients. After 40-fold concentration of 2 mL of CSF, 2 mg of immunoglobulin per liter can be detected, because the analytical sensitivity of the electrophoresis is 80 mg/L.

Additional Keyphrases: multiple sclerosis • silver-stain method compared

Electrophoretic detection of oligoclonal bands was originally described in cerebrospinal fluid (CSF) from multiple sclerosis (MS) patients (1–3). Although much of the work concerning oligoclonal banding has focused on the laboratory-assisted diagnosis of MS, these bands have also been reported in association with systemic lupus erythematosus

1 Department of Laboratory Service, Durham V.A. Medical Center, 508 Pulaski Street, Durham, NC 27705.
2 Department of Pathology, Duke University Medical Center, Durham, NC 27710.
3 Department of Hospital Laboratories, University of North Carolina, Chapel Hill, NC 27514.
4 Department of Clinical Neurological Sciences, Division of Neurology, University Hospital, London, Ontario, Canada N6A5A5.

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Oligoclonal Banding in Cerebrospinal Fluid Assessed by Electrophoresis on Agarose after Centrifugal Sample Concentration through a Microconcentrator Membrane

Robert H. Christenson,1,2 Mary E. Russell,1 Karen T. Guban,3 Lawrence M. Silverman,3 and George C. Ebers4

We describe use of a microconcentrator membrane with a 30,000-Da cutoff for treatment of cerebrospinal fluid (CSF) specimens before detection of oligoclonal bands by electrophoresis on agarose. After centrifugation at 2000 × g for 25 min, 0.5-, 1.0-, and 2.0-ml aliquots of CSF were concentrated 15-, 25-, and 40-fold. Analytical recovery of immunoglobulins G and A from the microconcentrators was about 90% (CV 5–8%). We found good correlation between results by this method and by a silver-stain procedure in a study comparing oligoclonal bands in CSF from multiple sclerosis and control patients. After 40-fold concentration of 2 mL of CSF, 2 mg of immunoglobulin G per liter can be detected, because the analytical sensitivity of the electrophoresis is 80 mg/L.

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Electrophoretic detection of oligoclonal bands was originally described in cerebrospinal fluid (CSF) from multiple sclerosis (MS) patients (1–3). Although much of the work concerning oligoclonal banding has focused on the laboratory-assisted diagnosis of MS, these bands have also been reported in association with systemic lupus erythematosus

8 Nonstandard abbreviations: CSF, cerebrospinal fluid; MS, multiple sclerosis; IgG, immunoglobulin G; IgA, immunoglobulin A.
(4), Guillain–Barré syndrome (5), polyneuropathy (5), pares-
thesia (5), aseptic meningoencephalitis (5, 6), and other disorders (7–11).

To aid in differentiating oligoclonal banding caused by intrathecal immunoglobulin production from that resulting from increased permeability of the blood–CSF barrier, it is necessary to electrophorese paired CSF and serum specimens simultaneously. Because various methods are used for quantification, the normal reference interval for IgG in CSF has not been clearly established. However, in a recent study (12), a normal reference interval of 8 to 42 mg/L is suggested for IgG in CSF, values that are below the analytical detection limits of conventional staining procedures. Thus CSF specimens must be concentrated before electrophoresis for study of oligoclonal banding when these gel-staining procedures are used.

Currently, most clinical laboratories concentrate CSF specimens by using devices in which absorbent pads, by wick action, draw water and permeating species through a selectively permeable membrane. Others use coloidal bags and systems involving removal of water and permeating substances through a filtering device by application of a vacuum. For the oligoclonal banding detection procedure we describe here, a centrifugal microconcentrator membrane (30 000-Da cutoff) is used to rapidly pre-concentrate immuno-
globulins in CSF specimens before electrophoresis on agarose. We assess the pre-concentration and electrophoretic procedures used in this method, to evaluate their use for oligoclonal banding detection. To verify the capability of the method in identification and differentiation of oligoclonal banding in positive and negative samples, we studied a series of CSF specimens from MS patients and controls.

Materials and Methods

Pre-concentration of samples. For concentrating samples and specimens we used Centricon-30™ microconcentrator devices (M, 30 000 cutoff, YM-type membrane) from Amicon Corp., Danvers, MA 01923.

Positive control. For use in preparing a positive oligoclonal banding control, we obtained sera from two IgG-type multi-
ple myeloma patients, each showing a distinct monoclonal protein that differed from the other in electrophoretic mobility on agarose. After measuring IgG by rate nephelometry (Beckman ICS; Beckman Instruments Inc., Brea, CA 92621), we exactly diluted each patient's serum to contain 40 mg of IgG per liter, using a prepared CSF-like matrix as diluent (11). To mimic the oligoclonal banding in each patient run, we aliquoted 1 mL of each CSF-like control into a microconcentrator device and treated it concurrently with the patients' CSF specimens.

Agarose electrophoresis. We used the Paragon™ SPE system with SPE-II precast gels and violet stain for electrophoresis, following the manufacturer's recommendations (Beckman Instruments Inc.).

Procedure. After centrifugation of the patients' CSF at 1000 × g for 10 min, either 1.0 mL (for 25-fold concentra-
tion) or 2.0 mL (for 40-fold concentration) of each CSF specimen was pipetted into Centricon-30 microconcentrator devices. All sample-containing devices, plus 1 containing a positive control, were then centrifuged (2000 × g, 25 min) in a 45° fixed-angle-rotor centrifuge. To recover the retained fluid ("retentate") containing the proteins of interest, we inverted the microconcentrator device and again centrifuged for 2 min at 1000 × g. We then applied 5 to 10 μL of the resulting retentate directly onto agarose gels. After allowing 5 min for diffusion of the sample into the agarose, the gels were electrophoresed for 40 min at 100 V. The sample-containing gels were then washed, fixed with a methanol/acetic acid solution, and treated with the violet stain to make protein visible. Oligoclonal banding was assessed as positive or negative by visual inspection of the sample gels.

Results

To determine the analytical detection limits of the agar-
ose electrophoresis method used in this method, we used a serum specimen from a patient with IgG-type multiple myeloma which contained 31.4 g of monoclonal protein per liter. Various dilutions of this IgG specimen were applied to agarose gels, electrophoresed, and stained, all as described above. Figure 1 shows the electrophoretic patterns corre-
sponding to these dilutions and their IgG concentrations. For those concentrations represented in lanes 1 through 6 of Figure 1, the monoclonal protein is easily visible, but less so for the IgG bands shown in lanes 7 and 8, depicting respectively 62.8 and 52.8 mg of monoclonal protein per liter. Because lane 6 of Figure 1 represents the lowest dilution of IgG that is visible, 78.5 mg/L, we conclude that the analytical sensitivity of this electrophoretic method is 80 mg/L. The normal reference interval for IgG in CSF report-
dly is 8–42 mg/L (12), below the concentration represented in lane 8 of Figure 1. Thus specimens submitted for oligo-
clonal banding studies must be concentrated before this and other conventional staining procedures are used.

To assess the concentrating ability and recovery of the Centricon-30 devices, we divided each volume of positive control shown in Table 1 among sets of four concentrating devices. After centrifuging these samples at 2000 × g for 25 min, we inverted the microconcentrators, and centrifuged again for 2 min at 1000 × g to collect the retained fluid. For each microconcentrator, the volume of retentate was determined gravimetrically; the concentration of IgG in each retentate was determined by nephelometry.

To calculate the volume concentrating ability of the devices, we used the following expression: (volume of posi-
tive control added to the microconcentrator)/(the retentate volume after centrifugation). As shown in Table 1, we found volume concentration factors of 13.4, 24.1, and 36.7-fold for the 0.5-, 1.0-, and 2.0-mL sample volumes, respectively.

The analytical recoveries for IgG that we observed for the

| Table 1. Precision and Recovery Data for the Centricon-30™ Microconcentrator Devices |
|-----------------------------------------|------------------------|----------|
| Vol of CSF-like material, mL | Mean retentate | Concen, fold | Anal. recov, IgG, % (and range) |
|---------------------------|----------------|-------------|
| 0.50                     | 32.4 (8.6%)    | 15.5        | 87 (78–93)          | 13.4 |
| 1.00                     | 34.6 (7.3%)    | 29.1        | 83 (76–88)          | 24.1 |
| 2.00                     | 49.8 (5.5%)    | 40.2        | 91.2 (88–94)        | 36.7 |

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Discussion

We found the reproducibility characteristics of the microconcentrators to be acceptable. No substantial difference in analytical recovery was found for IgA or IgG. Because IgM normally exists as a pentamer having a molecular mass of 900 000-Da, the permeability of the microconcentrator for this immunoglobulin will not be substantial.

References


Fig. 2. Typical electrophoreograms for MS-patients’ CSF samples (lanes 1–4) and control patients (lanes 5–7) Lane 8: positive control