Two Agarose Electrophoretic Systems for Demonstration of Oligoclonal Bands in Cerebrospinal Fluid Compared

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Demonstration of oligoclonal bands by electrophoresis of cerebrospinal fluid is an important aid in establishing the diagnosis of multiple sclerosis. Electrophoretic systems vary in their effectiveness in doing so. We compared two systems in this respect. For a thin-layer agarose system, sensitivity was less (47\%) than for a high-resolution agarose system (87\%). Each system had good specificity (92 and 85\%, respectively). Interpretation of electrophoretic patterns for cerebrospinal fluid should be available in clinical laboratories. Further, the best available system should be used for demonstration of oligoclonal bands.

Additional Keyphrase: multiple sclerosis

The role of the clinical laboratory in aiding in the diagnosis of multiple sclerosis has changed in recent years because of three procedures: demonstration of oligoclonal bands by agarose electrophoresis of cerebrospinal fluid (CSF) (1), quantitation of myelin basic protein in CSF (2), and quantitation of immunoglobulin G in CSF (3). The incidence of oligoclonal bands in the agarose electrophoretic pattern of the CSF of selected patients with demyelinating diseases is in the range of 70\% to 100\% (2, 4), although oligoclonal bands in the CSF are not specific for demyelinating diseases (1, 5). Methods used to demonstrate the oligoclonal bands may differ in sensitivity. Agarose is the electrophoretic support medium used most commonly, but the several commercially supplied variants of agarose electrophoretic techniques differ in many features. Previously, we assessed the performance characteristics of a commercial agarose electrophoretic system used to demonstrate oligoclonal bands (2). Here, we compare the performance of that system with that of a high-resolution agarose electrophoretic system.

Cellulose acetate is known to be an inappropriate support medium for the demonstration of oligoclonal bands (6).

Materials and Methods

Samples. Patients were selected only on the basis of availability of adequate CSF for examination by each method and availability of clinical history. Samples were obtained by standard lumbar puncture techniques.

CSF was concentrated 80-fold by selective permeability (Minicon CS 15; Amicon, Lexington, MA 02173).

Thin-layer agarose-gel electrophoresis. A 2-\(\mu\)L sample of the concentrate was applied, in two applications, to thin-layer agarose gel (Universal Electrophoresis Film; Corning Medical, Medfield, MA 02502). A 1-\(\mu\)L sample of each patient’s serum was applied to a position adjacent to his CSF sample. Elec-
Electrophoresis at pH 8.6 (Special Barbital Buffer; Corning Medical) was for 35 min at 20 mA (Electrophoresis Power Supply; Corning Medical). The thin-layer agarose gel was stained with Amido Black 10B, 2 g/L, for 10 min as soon as electrophoresis was completed. Excess stain was removed by immersing the gel in an acetic acid solution (50 mL/L), and the gel was then dried at 60 °C in an oven. For final clearing of the gel we used a second bath of 50 mL/L acetic acid; final drying was at 60 °C.

High-resolution agarose-gel electrophoresis. The concentrate was applied to the agarose gel (Panagel-8; Worthington Diagnostics, Freehold, NJ 07728) by use of a special mask supplied by the manufacturer. Excess sample was blotted off after 7 min. A serum sample from each patient was applied to the same gel in the same manner. CSF and serum samples were applied in sequence to different halves, such that the CSF and serum patterns could be stained separately after cutting the gel in half. Electrophoresis was for 45 min at 200 V (Panagel Migration Unit, Worthington Diagnostics) in a barbital buffer, pH 8.6 (Panagel Electrode Buffer, Worthington Diagnostics). The gel was fixed in a 110 g/L picric acid solution for 10 min, then washed in 95% ethanol for 3 min. The gel was pressed dry by applying lint-free absorbent paper, a glass plate, and a 1-kg weight for 20 min, then oven dried at 60 °C. Serum samples were stained with Amido Black as described above, except that final clearing was in de-ionized water. CSF samples were stained with Coomassie Blue R-250 (Eastman Kodak Co., Rochester, NY 14650) for 20 min, then cleared in a bath of 95% ethanol/glacial acetic acid/de-ionized water (25/10/65, by vol).

The dried, stained electrophoretic patterns were inspected separately and independently by two observers for the presence (positive) or absence (negative) of oligoclonal bands without knowledge of the patient’s identity, interpretation by the other system, or interpretation of the other observer. Patients were classified as having or not having multiple sclerosis according to the clinical impression of the managing physicians.

Sensitivity and specificity were calculated in terms of identifying the presence or absence of multiple sclerosis as defined previously (2, 7).

Results
CSF specimens from 110 patients were each electrophoresed by each method. The two observers consistently agreed in their interpretation of the patterns obtained with each system. On comparing the results of both systems, however, their interpretations were in agreement for only 98 specimens (89.1%). In each of the 12 cases of disagreement, their interpretation of the thin-layer pattern was negative, and that of the high-resolution pattern was positive. For the thin-layer system there were 14 interpretations of positive, and for the high-resolution system 26 interpretations of positive. Of the 12 specimens on which there was disagreement, six patients were considered by their physicians to have multiple sclerosis and six were not.

Multiple sclerosis was the clinical impression of the probable diagnosis in 15 patients. Of these, the thin-layer system identified seven (sensitivity = 47%), the high-resolution system 13 (sensitivity = 87%). Clinically, multiple sclerosis was not considered to be the diagnosis in 89 patients. Of these, the thin-layer system demonstrated oligoclonal bands in seven (specificity = 92%), and the high-resolution system demonstrated oligoclonal bands in 13 (specificity = 85%).

Discussion
As interest grows in laboratory procedures for aiding diagnosis of multiple sclerosis, it is important to evaluate the performance of these procedures. We reported elsewhere these characteristics for one agarose-electrophoretic method for demonstrating the presence of oligoclonal bands (2). Here, we demonstrate the high-resolution system to be more sensitive. The thin-layer system had a sensitivity of 77% in our previous study of 185 unselected patients (2). We cannot explain the markedly diminished sensitivity of 47% in this study, but in any case sensitivity of the high-resolution system (87%) exceeds both of these figures. Specificities of 95% for the thin-layer system (99% in our previous study) and 85% for the high-resolution system are good, especially if one considers that the patients were not selected on the basis of suspected multiple sclerosis. Obviously, patients from whom CSF is submitted to the laboratory are to some extent selected, in that some process that may involve the central nervous system is suspected. Specificity would be expected to be higher for a properly selected population.

The apparent “false positives” should not be viewed as necessarily diminishing the value of the procedure. Oligoclonal bands represent locally produced immunoglobulin of restricted heterogeneity (8). Oligoclonal bands are known to occur in the electrophoretic pattern for CSF of patients with neurosyphilis, Guillain-Barré syndrome, bacterial and viral encephalitis, and axonal polyneuropathy; in the proper clinical context their demonstration may be of value (2, 5, 9–11). The patients in this present study in whom oligoclonal bands were present but in whom multiple sclerosis was not considered at all likely included one each with Guillain-Barré syndrome, herniated intervertebral disc, diabetic polyneuropathy, and meningencephalitis. The others had neurologic abnormalities that were not considered to be manifestations of multiple sclerosis.

This study and the others cited demonstrate the value of CSF electrophoresis. It is important to recognize the marked difference in the ability of different systems to identify the presence of oligoclonal bands. Interpretation of CSF electrophoretic patterns should be routinely available in clinical laboratories that already are offering protein electrophoresis. The performance characteristics of a system must be considered when choosing a method to demonstrate oligoclonal bands, as their presence or absence is becoming more important in the classification and management of patients. We urge providers of laboratory services to use systems that have well-defined and acceptable performance characteristics.

References

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False-Negative Results for Urinary Phenothiazines and Imipramine in Forrest’s Qualitative Assays

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When a series of patients’ urine samples supplemented in vitro with chlorpromazine or imipramine was assayed with the Forrest qualitative assays, we observed an occasional false-negative result, which we found was attributable to interference by ascorbic acid. It interferes with the reagent, not with the analytes, in both assays. We easily eliminated this interference with the phenothiazine test by using an anion-exchange resin. Eliminating the interference with the assay for imipramine, however, is more difficult; false-negative results can be obtained even after ion-exchange chromatography if the imipramine concentration is <50 mg/L.

Forrest and Forrest (1) were the first to describe a simple, rapid, qualitative test for phenothiazines in urine involving use of ferric chloride, perchloric acid, and nitric acid (FPN). In 1961 Forrest et al. (2) reported on their use of the FPN screen for urinary phenothiazines and claimed that “virtually no false negatives have been encountered.” Campbell (3), however, in 1965 claimed more than 20% false-negative results with the FPN test for urinary phenothiazines. In 1966 Brownstein and Roberge (4) reported a false-negative rate of approximately 25% with the FPN test. Forrest et al. responded in 1966 (5), claiming that “…Campbell’s 20% false negatives were essentially authentic negatives, caused by drug-defaulters. …”

The Forrest FPN test has appeared unchanged in several recent texts on analytical toxicology (6) and clinical chemistry (7), and it is still generally accepted that a negative FPN test “effectively eliminates this group of drugs as a factor in diagnosing the condition of the patient” (7).

Forrest et al. (8) also developed a simple color test for imipramine in urine and attempted to use it to estimate daily drug dosage. This test also appears in a recent text (6) and is frequently included as part of a battery of tests for drugs of abuse.

We studied the Forrest tests on a series of patients’ urines supplemented in vitro with chlorpromazine or imipramine to a concentration of 50 mg/L. We found occasional urine samples that gave false-negative results. Subsequent investigation revealed ascorbic acid to be the interfering substance. This is a serious interference because of the many dietary sources of the vitamin and its frequent use in high doses as attempted therapy for infection of the upper respiratory tract.

Ascorbic acid also interferes with urinary glucose assays. A simple anion-exchange procedure has been reported that eliminates ascorbic acid interference with urine glucose procedures (9). We have modified the phenothiazine screening test (1) and imipramine screening test (8) to include an anion-exchange step before addition of color reagent. This procedure eliminates interference by vitamin C and the associated false-negative test results for phenothiazines; it also prevents interference with the Forrest test for imipramine if the urinary concentration of imipramine is at least 50 mg/L.

**Materials and Methods**

**Phenothiazines.** Prepare anion-exchange columns as follows. Weight 10 g of AG 1×4 anion-exchange resin, chloride form, 200–400 mesh (Bio-Rad Laboratories, Richmond, CA 94804) into a 125-mL Erlenmeyer flask. Add 35 mL of deionized water and stir. Add 0.5 mL of the anion exchange slurry into a Pasteur pipette containing a small cotton plug. This will produce an ion-exchange column that is about 1 cm × 4 mm i.d. Add 1.0 mL of the urine to be tested for phenothiazines to this ion-exchange column. Discard the eluate; it will contain no detectable phenothiazine but may contain small amounts of ascorbic acid, depending on the original ascorbic acid concentration in the urine. Add 1.0 mL of deionized water to the column and collect the eluate. This eluate will contain phenothiazine if present in the original urine sample. Add 1.0 mL of Forrest’s FPN reagent (1) to the collected eluate. A phenothiazine will be indicated by a pink or purple color.

**Imipramine.** The Forrest test for imipramine is much more sensitive to ascorbic acid interference than is the FPN test for phenothiazines. Use a 2 cm × 4 mm i.d. ion-exchange column to remove ascorbic acid from the urine sample. The procedure is the same as for phenothiazine except that 1.0 mL of the appropriate Forrest’s reagent (8) is added to 0.5 mL of the second eluate. A positive result for imipramine is indicated by a green color.

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