# Cerebrospinal Fluid Oligoclonal Bands in the Diagnosis of Multiple Sclerosis

## Isoelectric Focusing With IgG Immunoblotting Compared With High-Resolution Agarose Gel Electrophoresis and Cerebrospinal Fluid IgG Index

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## Abstract

*Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. We compared the diagnostic performance of isoelectric focusing (IEF) combined with IgG immunoblotting to high-resolution agarose gel electrophoresis for the detection of intrathecal synthesis of IgG due to the MS disease process.*

*In 20 patients with definite MS, IEF and high-resolution agarose gel electrophoresis had sensitivities of 90% and 60%, respectively. In the 51 patients with no evidence of MS, the methods had specificities of 94% and 96%, respectively. With a prevalence of 15% in this test population, IEF and high-resolution agarose gel electrophoresis had positive predictive values of 73% and 73% and negative predictive values of 98% and 93%, respectively.*

*The IEF method improves the sensitivity and the negative predictive value of the oligoclonal-banding assay, the IEF gels are easier to interpret, and the IEF assay requires a smaller cerebrospinal fluid volume.*

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. Cerebrospinal fluid (CSF) is used in the diagnosis of MS to identify intrathecal IgG synthesis[1] as reflected quantitatively (IgG index or IgG synthesis rate) or qualitatively (oligoclonal bands [OCBs]).[2] The finding of OCBs in CSF and not in the serum supports the diagnosis of MS.

The CSF IgG index is elevated in up to 90% of patients with MS.[3] Detection of OCBs by agarose gel electrophoresis (AGE) has been reported to have a sensitivity of 45% to 74% and a specificity of 95% to 97% in definite MS cases.[3,4] Newer OCB detection methods such as isoelectric focusing (IEF) and immunoblotting have been reported to be more sensitive for OCB detection in patients with MS.[4,5] We compared the performance of AGE and IEF assays for OCBs in a panel of subjects with and without MS to define the positive and negative predictive values of the methods.

## Materials and Methods

### Samples

We analyzed paired samples of CSF and serum obtained from 175 patients who attended the Department of Neurology, Mayo Clinic, Rochester, MN. The samples were submitted to the clinical laboratory for analysis of oligoclonal IgG bands and determination of the CSF IgG index. A minimum risk protocol was obtained from the Mayo Clinic Institutional Review Board to review patient histories. The diagnosis of definite MS was established according to the Poser criteria.[6] Other diagnostic groupings represent patients

classified as having possible or probable MS, non-MS with an inflammatory neurological disorder, and a noninflammatory neurological disorder. The four groups were classified independent of the CSF results.

The CSF and serum paired samples were collected and analyzed for OCBs by AGE and the IgG index was determined on the same day as specimen collection. The paired samples subsequently were stored at –20°C for testing in the IEF assay for OCBs.

### Detection of OCBs

AGE was performed on CSF samples concentrated 40-fold.[7] The paired CSF and serum samples were run on the same gel (Helena Laboratories, Beaumont, TX) to permit determination of unique bands in the γ region of the CSF. A 4.0-μL aliquot of serum, prediluted 1:6 with high-resolution buffer, and 4.0 μL of concentrated CSF were applied to the gel. Electrophoresis on the high-resolution agarose gel was performed for 20 minutes at 250 V. Coomassie brilliant blue was used as the stain. A positive test was considered as 2 or more bands in the γ region of the CSF that did not appear in the serum on the electrophoresis pattern. Two technicians independently reviewed the results. In 5% to 10% of the samples, the technicians were not in agreement. A third technician resolved the difference.

The agarose gel IEF with IgG immunoblot assay used gels and reagents from Helena Laboratories. In this assay, 5.0 μL of serum prediluted 1:300 and 5.0 μL of unconcentrated CSF were applied. Electrophoresis was performed on agarose IEF gel, pH 3 to 10, for 35 minutes at 800 V and 10°C. Following electrophoresis, protein was transferred passively onto a nitrocellulose membrane, and IgG was detected by addition of sheep antihuman IgG peroxidase conjugate.[8] A positive test was considered as 4 or more bands in the CSF that did not appear in the serum pattern. The use of 4 or more bands gave the best separation between MS and non-MS cases in this study.

The results were reviewed independent of the high-resolution agarose electrophoresis results. A control sample was assayed on 29 separate IEF gels to determine the interassay precision. The results were 4 or 5 bands representing a variation of ± 0.5 OCBs. Sensitivity was determined by serially diluting positive CSF samples. Bands could be detected in these samples to a dilution of 0.525 mg/dL of IgG. Among the samples saved in anticipation of this study, 98.2% (388/395) of samples had a concentration greater than 1 mg/dL of IgG, and the lowest IgG concentration was 0.7 mg/dL. The CSF and serum samples were stable for 7 days at ambient temperature, 14 days refrigerated, 3.5 months frozen at –20°C, and for 3 freeze-thaw cycles. The IgG IEF gel blots also were read independently by 2 observers (E.L.S., J.A.K.). The only discrepancies were in number of bands in clearly positive CSF with 8 or more bands. There were no differences regarding classification of normal and abnormal.

### IgG Index

The quantitative determinations of albumin and IgG in the serum and in CSF samples were measured by nephelometry. We used the IgG index obtained by dividing the CSF/serum IgG concentration ratio by the CSF/serum albumin concentration ratio; the reference range for the CSF IgG index is less than 0.85.

## Results

To gain experience with the IEF and immunoblotting of CSF and serum samples, a comparison of AGE and IEF for OCBs was performed in 59 paired CSF and serum samples. These samples were chosen to represent 25 samples positive for OCBs and 34 samples negative for OCBs as determined by AGE. The clinical diagnosis was unknown for this group of 59 patients. There were 20 samples that were positive in both assays and 31 that were negative in both assays (86% concordance). Among the 8 discordant results, 5 samples were positive by AGE and negative by IEF, and 3 samples were negative by AGE and positive by IEF. A number of observations were apparent during this initial comparison. First, the OCBs were sharper and easier to detect with the IEF assay. In addition, among the 31 concordant negative samples, 19 had 1 band by AGE and no bands by IEF, whereas among the 20 concordant positive samples, the average number of bands was 2 by AGE and 8 by IEF. The IEF assay was, therefore, simpler to interpret, and the distinction between negative and positive results was clearer on IEF (0 vs 8 bands) than with AGE (1 vs 2 bands).

We assessed sensitivity by selecting a group of 20 cases definitely diagnosed as MS. In this group, positive OCBs were detected in 12 cases by using AGE and 18 patients by using IEF. The CSF IgG index was elevated abnormally in 14 of 20 cases **Table 1**. Scans of representative gels are illustrated in **Image 1**. The CSF and serum samples from each of 3 patients were tested by AGE and IEF. On the AGE gel, the OCBs migrated in the γ region and were faint and fuzzy. On the IEF gel, the OCBs distributed along the pH gradient and were sharper and easier to detect (Image 1).

We assessed specificity by selecting 51 consecutive paired samples from patients with no evidence of MS. To obtain 51 consecutive patients, we needed to capture 60 consecutive CSF-serum pairs. Among these 60 cases, there were 9 patients with MS. Assays on the samples from the non-MS cases indicated that the AGE and the IEF had




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**Table 1**
**Sensitivity Study for 20 Definite MS Cases and Specificity Study for 51 Non-MS Cases***

| Method | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| Oligoclonal bands | | | | |
| Isoelectric focusing | 90 | 94 | 73 | 98 |
| Agarose gel electrophoresis | 60 | 96 | 73 | 93 |
| CSF IgG index | 70 | 96 | 76 | 95 |

CSF, cerebrospinal fluid; MS, multiple sclerosis; NPV, negative predictive value; PPV, positive predictive value.
* Data are given as percentages. PPV and NPV assumed a prevalence of 15%.

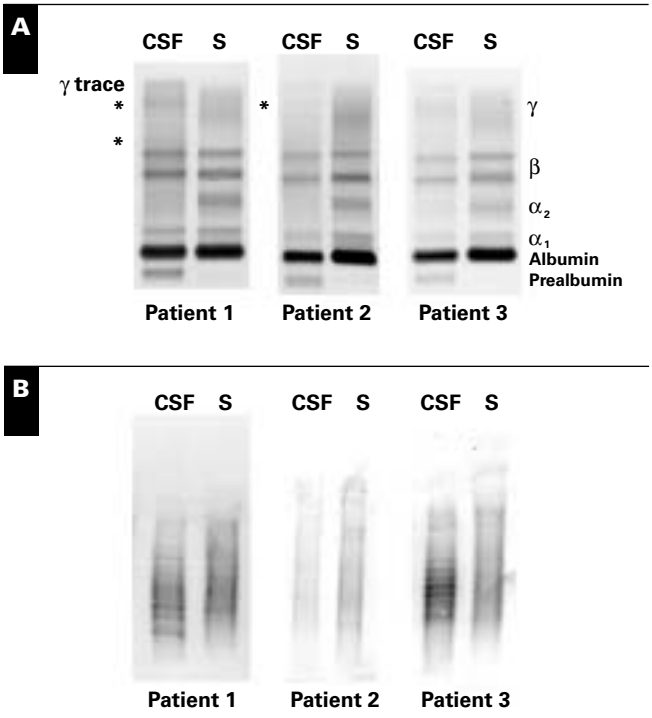


**Image 1** Representative gel scans of the high-resolution agarose electrophoresis (**A**; AGE) and IgG immunoblot isoelectric focusing (**B**; IEF) methods. For each of 3 patients, cerebrospinal fluid (CSF) and serum samples were tested by both methods. The oligoclonal bands (OCBs) on the AGE are denoted by asterisks. These bands are fuzzy and faint. Patient 1 has a diagnosis of multiple sclerosis (MS) and positive results in both assays: 2 OCBs in CSF by AGE and 8-10 OCBs by IEF. Patient 2 does not have MS and has negative results for both assays: 1 faint CSF band by AGE and no bands by IEF. Patient 3 has a diagnosis of MS and has discordant OCB results: no bands by AGE but 8 OCBs in CSF by IEF.

specificities of 96% and 94% respectively (Table 1). There were 36 non-MS cases that had 1 band on the AGE and no bands by IEF, confirming the original observations of fewer OCBs detected by IEF in negative cases. These 51 patients had other nervous system disorders that included axonal sensory motor peripheral neuropathy, dementia, hereditary spastic paraparesis, juvenile myoclonic epilepsy, spinocerebellar ataxia, and transient ischemic attack. The specificity of the CSF IgG index was 96%.

The false-positive results in this group were the following cases: (1) a patient with presumed autoimmune encephalopathy who had positive results in all 3 assays, (2) a patient with meningitis with positive IEF results for OCBs and a positive CSF IgG index, (3) a positive AGE result for a patient with migraines, and (4) a positive IEF result for a patient with lymphoma with spinal cord compression. Therefore, the 3 non-MS cases with positive results in the IEF assay had diagnoses of autoimmune encephalopathy, meningitis, and lymphoma with spinal cord compression, whereas those with positive AGE results included autoimmune encephalopathy and migraine.

Among the 60 consecutive cases, 9 were MS cases. This suggests that among the samples being tested in our laboratory for OCB, there is a 15% prevalence of MS. By using this prevalence and the sensitivities and specificities already described, we calculated positive and negative predictive values (Table 1). Because most samples are from non-MS cases, the small decrease in specificity has a large effect on the positive predictive value, and the positive predictive values for both OCB assays are identical despite the large increase in sensitivity of the IEF assay. The increased sensitivity of the IEF assay, however, enhances the negative predictive value of the test.

Among the 20 definite MS cases tested by IEF, 17 had at least 8 OCBs, 1 had 4 OCBs, and 2 had fewer than 4 OCBs. The distribution of banding results in a number of other diseases that were selected because of elevated CSF IgG index results is given in **Table 2**.

## Discussion

Many laboratories have used high-resolution AGE for the identification of OCBs in CSF samples from patients with MS. We have routinely used AGE and 40-fold concentrated CSF samples. The IEF procedure used in this study uses unconcentrated CSF samples and an IgG immunoblot for the detection of CSF-specific OCBs. IEF assays have been reported to be more sensitive and less specific for detecting OCBs.[5,9]

The bands in the IEF immunoblot assay were sharper and easier to identify than in the AGE assay. In addition, there were fewer equivocal results. That is, among the 31 cases in the initial comparison study with negative results of both OCB assays, 19 had 1 band by AGE and no bands by IEF. Conversely, among the 20 cases with positive results by both OCB assays, the mean number of bands in the AGE assay was 2 compared with 8 in the IEF assay. The ease of identifying the bands and the larger separation between


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**Table 2**
**Number of Oligoclonal Bands by IEF in CSF of 59 Patients With Different Neurological Manifestations**

| | No. of Bands in CSF | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Clinical Diagnostic Group** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **≥8** |
| Definite MS (n = 20) | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 17 |
| Possible or probable MS (n = 6) | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| Non-MS, inflammatory (n = 15) | 6 | 1 | 1 | 2 | 0 | 0 | 0 | 2 | 3 |
| Non-MS, noninflammatory (n = 18) | 8 | 2 | 2 | 1 | 0 | 0 | 0 | 2 | 3 |

CSF, cerebrospinal fluid; IEF, isoelectric focusing; MS, multiple sclerosis.

negative and positive results makes the IEF assay much easier for the clinical laboratory.

Reviewing the banding results for the series of MS and non-MS cases led us to conclude that 4 or more CSF-specific bands should be considered the diagnostic range in the IEF assay compared with 2 or more bands in the AGE-OCB assay. By using a cutoff of 4 or more CSF-specific bands for IEF and 2 or more CSF-specific bands for AGE, the IEF assay showed a substantial increase in sensitivity (90%) compared with AGE (60%), as other investigators had noted.[2-5] This increase in sensitivity is offset by a small decrease in specificity from 96% to 94%.

There are other neurologic diseases besides MS that can be expected to generate humoral immune responses resulting in CSF-specific OCBs. These diseases include infections and inflammatory, cerebrovascular, and paraneoplastic disorders.[4,10] Among the 4 non-MS cases in our specificity study that showed positive results in at least 1 assay, 2 are in this "inflammatory" group: 1 case of autoimmune encephalopathy and 1 case of meningitis.

Several of the 5 cases in the non-MS, noninflammatory group in Table 2 with 7 or more CSF-specific bands for IEF had nonspecific abnormalities detected by magnetic resonance imaging and, in some cases, symptoms such as paresthesias that could be compatible with MS. Although a diagnosis according to accepted criteria was not possible, they could have mild or subclinical MS.

As this study shows, IEF improves the performance of the test by easier recognition of OCBs and improves the sensitivity and the negative predictive value by 30% and 5%, respectively. In contrast, the specificity is decreased by 2%. Although no current laboratory assays are specific for MS, the IgG-IEF method seems to be superior to high-resolution agarose OCB assays in diagnostic testing for MS.

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