Letters to the Editor should be typed doubled-spaced (including references) with conventional margins. The overall length is limited to five manuscript pages, including not more than one figure or one table.

Oligoclonal Bands Are Found in Electrophoreograms of Serum of Patients with Multiple Sclerosis

To the Editor:

A recent paper (1) describing oligoclonal banding in CSF indicates that it is necessary to electrophorese paired CSF and serum specimens simultaneously, to differentiate oligoclonal banding caused by intrathecal immunoglobulin production from increased permeability of the blood–CSF barrier. This dogma is not correct, as it has been shown by use of polyacrylamide gel isoelectric focusing and Coomassie Brilliant Blue staining or agarose gel electrophoresis that 74% and 9%, respectively, of patients with multiple sclerosis having oligoclonal bands in the CSF patterns also had bands in the corresponding patterns for serum (2). These bands were not seen in patterns for normal CSF and normal serum (2).

We have investigated the frequency of oligoclonal banding in sera of patients with multiple sclerosis by using agarose isoelectric focusing, staining with silver. In our group of 12 patients with multiple sclerosis, bands were present in the sera of 11. Paired electrophoreograms of CSF and serum showed that the oligoclonal bands in both had identical electrophoretic mobilities.

Just why the oligoclonal bands are present in the patterns for sera of patients with multiple sclerosis has not been determined, but if the criterion of oligoclonal bands in CSF and not in serum is used to determine clinical significance, this may lead to false-negative results.

References

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Modifications in a Method for Vanillylmandelic Acid in Urine

To the Editor:

We recommend several modifications to a recently published (1) method for determination of vanillylmandelic acid (VMA) in urine. Although the method usually works well as described, we and others (personal communications, 1985) noticed batch-to-batch variations in the analytical columns, in terms of efficiencies (plate count), retention times, and longevity. Because good efficiency and reproducible selectivity is required for VMA to be resolved from earlier-eluting peaks, we now use a 4.6 × 150 mm column of 5-µm Altex Ultrasphere C18 for the reversed-phase analytical separation.

We use mobile phase consisting of 100–150 mL of methanol and 850–900 mL of phosphate buffer (pH 3.5, 0.1 mol/L) per liter, with 2 mmol of Q3 (Regis Chemical Co. brand of N-triethyl-octylammonium phosphate) ion-pair agent per liter. This mobile phase differs from that described previously only in that 100 to 150 mL of methanol is used per liter instead of 40 mL. Retention times for VMA and isovanillylmandelic acid are 4.0 and 5.6 min, respectively, when a 150 mL/L methanol mobile phase is delivered at 1.5 mL/min.

Several commercially available 10-µm C18 reversed-phase packings should be suitable for packing the 5 × 30 mm minicolumns used for the preliminary purification of urine samples. Based on their retention characteristics, the following are possible alternatives to the 10-µm Bondapak packing previously used to pack the minicolumns: Nucleosil C18, Supelcosil C18, Hypersil SAS, Partisol ODA, and Brownlee RP18 (LiChrosorb). We tried several solid-phase absorption cartridges for the preliminary purification step but none sufficiently retained late-eluting peaks.

We continue to use the method, with no problem other than the aforementioned inconsistencies of the analytical columns, and we can now report a day-to-day CV of 4.9% (6.60 ± 0.32 µg/mL, n = 14) for aliquots of a quality-assessment sample tested during 10 months.

References

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Poor Performance of the Baker Nephelometer and Reagents

To the Editor:

We would like to report our experience with and problems in the use of the Baker 420 laser nephelometer and its reagents (Baker Instruments Corp., Allentown, PA 18103) for measurement of specific proteins. This system assays proteins and drugs by monitoring polymer-enhanced antigen–antibody complex formation at antibody excess, by use of kinetic or end-point nephelometry. Our problems surfaced soon after installation of the instrument (serial no. 249) and test runs in April 1984, when most of the manufacturer's antisera to IgG, IgM, IgA, C3, C4, haptoglobin, and a2-macroglobulin exhibited background light scattering 1.6 to 5.0 times above the maximally allowable QC limit of 150 light scattering units. Unacceptably high back-
ground light scattering occurred regardless of whether the antisera were clear or visibly cloudy, while the instrument's "QC REAGENTS" procedure for all the buffer and polymer solutions gave satisfactory results.

We then followed the manufacturer's recommendation (1) to filter the antisera: initially through Millipore 0.2- or 0.5-$\mu$m polytetrafluoroethylene membrane and subsequently, for fear of nonspecific adsorption of protein, through a special membrane provided by Baker. All ultrafiltrates showed background light scattering well below the nephelometer's quality-control limits. They could always be used in conjunction with the Baker 420 Reference A serum to generate six-point reference curves for the designated antisera covering low, normal, and high concentrations. But not infrequently, the highest reference point had to be deleted for acceptable curve fitting, so that the resulting calibration had five points and covered only modestly high concentrations. In any case, analysis of Baker 420 Immune-C control serum for IgG, IgA, IgM, C3, C4, haptoglobin, and $\alpha_1$-macroglobulin (at mid-normal concentrations) gave results that were accurate and precise.

However, two problems were identified during further evaluation of the instrument. Firstly, there were too frequent "HIGH" messages and too many "REDILUTE" steps in the analysis of patient sera, especially when the five-point reference curves were used. A similar problem was encountered with Reference A and Immune-C sera when they were diluted less (with Baker 420 buffer solution) to give high concentrations. The design of the nephelometer is such that if the peak rate of a sample exceeds 2.7.3.2) the highest reference point in the reference curve, a "HIGH" message is displayed. The instrument can be programmed to immediately retest the sample, using half of the original volume of 20 or 35 $\mu$L. This process is repeated until the peak rate falls within the reference curve or until sample volume of 3 $\mu$L is reached. Thus the filtered antisera behaved as if they could only maintain antibody excess not far beyond—or sometimes below—the highest reference point in the reference curve, so that additional costly time-and-reagent-consuming redilution steps were required to assay even modestly high samples.

Secondly and more importantly, using Reference A and Immune-C sera reconstituted to give different concentrations, we found that assays with filtered antisera became nonlinear at antigen concentrations beyond about two times the upper limit of their respective normal ranges. For example: serum IgG assay (normal range 654-1430 mg/dL) was nonlinear beyond 3200 mg/dL, although Baker's claimed linearity (2) was up to 12 800 mg/dL; serum C3 assay (64-140 mg/dL) was nonlinear above 218 mg/dL when the claimed limit was 3456 mg/dL (3). This happened with all the seven assays tested, and replacement of buffer with 150 mmol/L NaCl solution for serum pre-dilution brought no improvement. Based on these difficulties it is obvious that assays done with the nephelometer using filtered antisera would be slow, costly, and unreliable for a significant proportion of patients' specimens in which the specific protein concentrations are high.

Over the last 14 months Baker Instrument Corp. and its local agent have tried without success to rectify the problems. The nephelometer is simple and easy to operate. Testing with a latex-particle suspension suggested that its light-scattering detection and recording were not at fault, and we have verified that the problems were not analyst dependent. Ultrafiltration of antisera, as recommended by the manufacturer, should be a logical remedy to background turbidity if it is nonspecific. In our experience, volume recovery was good (85-90%) and total protein concentration decreased by 8 to 15% from the original values of 15 to 25 g/L. The Operator's Manual does mention that high light scattering of antisera may occur occasionally. However, such frequent occurrence and its undesirable consequences were unexpected. We are not sure of the exact cause, but one speculation is antiserum deterioration during shipment. Adverse conditions such as prolonged delay in delivery, exposure to heat, or drastic changes of temperature may have visibly or invisibly precipitated much of the antibodies, resulting in their removal by ultrafiltration. Consequently, antibody concentration and titer of the filtered antisera could have become so low that they could not be used to measure the concentrations of specific proteins usually encountered. We have been forced to resume the use of radioimmunodiffusion to sustain our specific-proteins service during this period. To date there has been no prospect of a solution, and we wonder whether other laboratories, particularly those outside the U.S., have encountered a similar problem.

References

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A spokesman for Baker responds:
To the Editor:

After evaluating this particular Series 420 Laser Nephelometer's performance through several troubleshooting protocols, we have verified that this particular system is not functioning according to our specifications. We are now making arrangements to rectify this problem with Drs. Lam and Swaminathan.

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Interference by Macro Creatine Kinase Type 1 with an Immunoenzymometric Method for Quantification of CK-MB in Serum

To the Editor:

We recently described (1) a case in which a macro creatine kinase (CK; EC 2.7.3.2) type 1 mimicked CK-MB isoenzyme in electrophoresis on agarose gels (Corning Medical, Medfield, MA). The "QuiCK-MB" (International Immunoassay Labs, Santa Clara, CA) and "Tandem-E CK-MB" (Hybritech, Inc., San Diego, CA) immunoassays yielded normal mass concentrations of CK-MB in the serum of this case as well as in four additional patients whose sera contained macro CK type 1 without CK-MB isoenzyme. Similar specificity of results has been described by Allen et al. (2), using the Tandem-E CK-MB method, for four patients' sera containing macro CK type 1 and no CK-MB. We describe here a case of interference with the Tandem-E CK-MB assay by a macro CK type 1.