

NEQAS analysis, and that any differences in detection rate of unsatisfactory performance were not attributable to the method of statistical analysis (5).

The UK NEQAS has so far not provided the requisite 5 CoaguChek-dedicated test samples in a single exercise. Combining results of serial exercises performed over a period of months or years as detailed in the report by Kitchen et al., with nonspecific INR, is an understandable but less reliable approach to EQA of the POCT PT monitors. Thus the smaller number of unsatisfactory performances detected by UK NEQAS compared with ECAA/EAA studies and the lower detection rate of unsatisfactory CoaguChek test strips is easily explained.

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Interlaboratory Reproducibility of Isoelectric Focusing in Oligoclonal Band Detection

To the Editor:

Current criteria for the diagnosis of multiple sclerosis (MS), an inflammatory neurological disease commonly affecting young adults, include cerebrospinal fluid (CSF) analysis to detect oligoclonal IgG bands (OCB) (1). CSF analysis methods vary substantially, however, and experts in MS and CSF diagnostic techniques addressed the need for standardization led by compiling recommendations (2). External quality control schemes are fundamental steps in standardization processes, particularly in the field of isoelectric focusing (IEF), the recommended technique for OCB detection (1–4),

because many IEF steps may be difficult to standardize (5).

Data from our previous OCB quality control survey showed that participating centers concurred in OCB-positive and OCB-negative sample identification, but differed in the numbers of OCBs found (5). We assumed that this lack of reproducibility could lead to false-negative/positive results in critical CSF samples, i.e., samples with few and weak bands. Accordingly, we aimed to produce a more comprehensive survey by involving more centers and by including critical samples.

We asked the 20 laboratories that participated in the 2006 OCB Quality Control Survey performed by the Italian Association for Neuroimmunology to blindly analyze freshly collected paired CSF and serum samples from 4 patients (controls A–D) with clinically isolated syndrome, a disorder that converts into MS in ~50% of cases (1). IgG concentrations in the samples were provided. Laboratories were asked to interpret the IEF, and to report the number of bands observed. All participants used IEF with immunoblotting for IgG, in accordance with recommended procedures (2, 3, 5). IEF was performed with agarose/polyacrylamide gels from the following suppliers: Helena (n = 9), homemade (n = 4), Pharmacia (n = 3), Amersham (n = 2), Sebia (n = 1), and Cambrex (n = 1).

Results for control A were OCB-negative in 15 centers, and OCB-positive with a mirror pattern (i.e., identical OCB in CSF and serum) (4) in 5 centers. All 20 centers identified CSF OCB in controls B and C, but additional serum bands were found in controls B (12 centers) and C (8 centers). For control D, 13 centers found a few CSF bands, the remaining centers found none; control D was accordingly considered a critical sample. Minimum and maximum (median) band numbers in control samples were as follows: A [0–6, (0), serum; 0–6, (0) CSF], B [0–15, (3); 3–26, (13)], C [0–8 (0); 5–20, (9)], D [0–5, (0); 0–7, (2)] (Kappa statistic for interobserver agreement was not significant for each control). Fig. 1

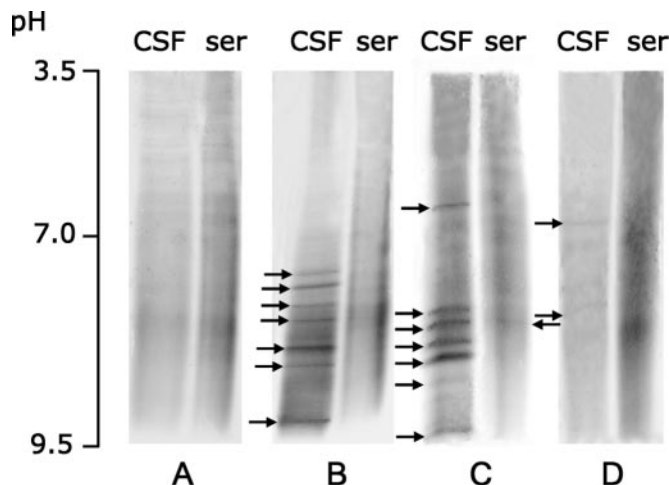


Fig. 1. Paired CSF and serum (ser) samples used as controls (A–D) in the survey, as analyzed by one center.

IEF was in agarose gel and staining of nitrocellulose paper after affinity-mediated protein blotting was IgG-specific. Arrows indicate OCBs

shows the 4 controls as detected in the center whose reported band numbers were the nearest to the median values in each control.

The main finding of the survey was the unacceptably large interlaboratory variation not only in OCB numbering, as already ascertained in 2005 (5), but also in qualitative reporting of the OCB pattern and in differentiating OCB-positive from OCB-negative samples. Misinterpretation of control A as a mirror pattern leads to a misleading suspicion of systemic inflammation/disease. Similarly, but with less impact on diagnostic workup, the exceedingly high number of serum bands found by some centers in controls B and C indicates acute, rather than chronic (as in the case of OCB unique to CSF) inflammatory disorders of the central nervous system. More important was the variation in OCB identification in control D, with 65% of survey responses indicating OCB-positive and 35% OCB-negative results; such variation is important for the correct diagnosis of MS.

The participation of new centers that lack specialized sections for CSF analysis [general laboratories (GL)] probably accounts for the worsening in results between the 2005 (2 of 11 GLs) and 2006 (8 of 20 GLs) surveys. The interlaboratory differences were not gel dependent

(data not shown). Possible remaining causes include (a) misinterpretation of artifactual bands, which derive from a nonhomogeneous pH gradient, as true bands (OCB-negative controls should help identify gradient-dependent bands), which would yield erroneous mirror patterns and exceedingly numerous OCB; (b) insufficient IEF skills, which could lead to underestimation of OCB (OCB-positive and hemoglobin controls should be built into IEF protocols); and (c) poor blotting and staining skills, which could distort OCB interpretation.

Our findings indicate that CSF analysis for OCB detection should be performed by experienced laboratories carefully selected by neurologists (2). Inadequate technical training and/or neurological background seem to be substantially responsible for unreliable OCB detection. Analysis of CSF samples with few and weak bands is absolutely critical and may yield false-negative results, even in experienced laboratories. Through educational support and external quality control schemes, scientific associations involved in CSF analysis play essential roles in promoting quality.

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Serum and Plasma Cardiac Troponin I 99th Percentile Reference Values for 3 2nd-Generation Assays

To the Editor:

Clinical and laboratory medicine associations have endorsed the implementation of the 99th percentile ref-