Comparison of Methods for the Detection of Oligoclonal IgG Bands in Cerebrospinal Fluid and Serum: Results of the Dutch Quality Control Survey, *Marcel M. Verbeek,[1,2*] Herman P.M. de Reus,[2] and Cas W. Weykamp[3]* ([1] Department of Neurology and [2] Laboratory of Pediatrics and Neurology, University Medical Center, 6500 HB Nijmegen, The Netherlands; [3] Stichting Kwaliteitsbewaking Ziekenhuis Laboratoria, PO Box 9005, 7100 GG Winterswijk, The Netherlands; * address correspondence to this author at: University Medical Center Nijmegen, Department of Neurology, 319 LKN, PO Box 9101, 6500 HB Nijmegen, The Netherlands. Fax 31-24-3540297, e-mail m.verbeek@cukz.umcn.nl)

Detection of oligoclonal IgG bands (OCBs) in parallel cerebrospinal fluid (CSF) and serum samples is an important tool to support the diagnosis of neuroinflammatory diseases such as multiple sclerosis (MS) *(1)*. Because ~90–95% of the patients diagnosed with MS present with OCBs in the CSF *(2)*, whereas other CSF indices may be normal, this type of analysis has long been recommended for the diagnosis of MS, especially when the clinical presentation does not clearly suggest this diagnosis *(1, 3)*. CSF-restricted OCBs are also essential for the diagnosis of primary progressive MS *(1)*. Furthermore, in several neuroimmunologic disorders, including neuroborreliosis, neurosyphilis, and viral infections of the brain, the identification of OCBs in CSF may be helpful in the diagnostic process *(2, 4, 5)*.

Twice a year, pairs of CSF and serum samples are distributed by our laboratories to the participants of the Dutch CSF Quality Control survey for analysis of OCBs. It was the aim of this study to evaluate the performance of the 56 participating laboratories for OCB analysis with regard to both the methods used for protein separation and detection of OCBs and to derive a general recommendation on laboratory management for this type of analysis.

To collect a sufficient volume of CSF, samples stored at −80 °C and selected for the absence of OCBs were pooled. Sera taken from healthy controls were collected and pooled as well. Sera containing either a known OCB pattern or monoclonal IgG were also collected with informed consent from patients. We added minimal amounts of these clinical serum samples to the CSF pool to create artificial patient CSF samples. In each survey, an OCB-enriched CSF sample and a serum sample were sent out as a pair of clinical CSF and serum samples. In this way we were able to create OCB patterns with variable intensities and patterns. The IgG and total protein concentrations in both the CSF and serum were made available to the participants. Participants were requested to analyze the OCBs in both the CSF and serum samples and to interpret the results according to one of the typical types of OCB patterns *(6)* (Table 1) and report on the method they used.

Isoelectric focusing (IEF) in combination with immunoblotting *(2)* was used as the standard method in the reference laboratory. CSF and serum samples, either containing 100 ng of IgG, were loaded, and IEF was performed for 1 h at 1000 V. After passive transfer of the proteins to a nitrocellulose membrane, the membrane was incubated successively with 20 g/L low-fat milk powder in 9 g/L NaCl, goat anti-human IgG Fc (Diasorin), and peroxidase-labeled rabbit anti-goat antibodies (Dako). The IgG was then visualized by the addition of 3-amino-9-ethyl-carbazole as chromogen in the peroxidase reaction (examples of the resulting OCB patterns can be seen in Fig. 1, which is available in the data supplement accompanying the online version of this Technical Brief at http://www.clinchem.org/content/vol48/issue9/). Results from the participants were compared with this "gold standard" *(7)*. The reference laboratory participates in a German quality-control survey for the detection of OCBs. The number of participants reporting results varied between 47 and 56 for each survey.

Table 1. Comparison of the results for the detection and interpretation of oligoclonal IgG bands in CSF and serum: EPS vs IEF, subdivided by detection methods.

		Correct results, % (no. of participants)				
Case	Correct type[a]	EPS + direct stain	EPS + immunofixation	EPS + PVDF blot + gold stain	IEF + direct stain	IEF + immunoblot
IV	1	91 (11)	100 (11)	75 (12)	80 (5)	100 (7)
V	2	44 (9)	78 (9)	71 (14)	67 (6)	100 (7)
VI	4	0 (10)	0 (9)	38 (13)	17 (6)	86 (7)
VII	4	11 (9)	0 (7)	13 (15)	25 (4)	86 (7)
VIII	1	100 (9)	100 (7)	80 (15)	80 (5)	100 (7)

[a] Types of OCB patterns: 1, normal (no OCBs); 2, CSF-restricted OCBs; 3, CSF-restricted OCBs with additional, identical bands in CSF and serum; 4, identical OCBs in CSF and serum; 5, monoclonal bands in CSF and serum.

The complexity of each task was classified by the reference laboratory as "easy" (e.g., absence of OCBs in both serum and CSF, distinct OCBs with high intensities, or monoclonal IgG), "moderate" (distinct OCBs with moderate intensities), or "difficult" (distinct OCBs with low intensities).

Several methods were used by the participants. Basically, these could be separated into two groups: those using electrophoresis (EPS) and those using IEF. In the EPS group, detection was performed by one of the four following methods: (*a*) direct protein stains (either silver or gold; n = 9–15, depending on the number of responders); (*b*) immunofixation with anti-IgG antibodies (n = 7–11); (*c*) blotting to nitrocellulose membranes and subsequent immunodetection with anti-IgG antibodies (immunoblotting; n = 1–4); or (*d*) transfer to polyvinylidene difluoride (PVDF) membranes after high-resolution EPS and staining with gold (n = 11–15). IEF was combined with (*a*) direct protein staining (silver or gold; n = 4–6), (*b*) immunofixation (n = 0–1), (*c*) blotting to nitrocellulose and protein staining (n = 0–4), or (*d*) immunoblotting (n = 5–7). Comparison were made between the entire EPS and IEF groups and among the major subgroups.

The results for the analysis of OCBs in eight paired CSF/serum samples by either the EPS or IEF groups are

given in Table 1 of the data supplement accompanying the online version of this Technical Brief at http://www.clinchem.org/content/vol48/issue9/. In general, in case of a type 1 (normal) pattern (cases II, IV, and VIII), the performance of either group was comparable (percentage of correct results: EPS, 80–92%; IEF, 81–93%). However, in four cases (I, V, VI, and VII), the IEF group performed much better than the EPS group. This was especially evident in difficult tasks, such as the detection of identical OCBs in CSF and serum (type 4; cases VI and VII), where only 9% and 14% correct results were reported in the EPS group, whereas the IEF group reported 42% and 50% correct results, respectively. In addition, in the cases in which CSF-restricted OCBs had to be detected (type 2; cases I and V), the IEF group performed better (73% and 86% correct results, respectively) than the EPS group (36% and 68% correct results, respectively). This difference was even more evident in the easy case (case I), which had a high concentration of OCBs, than in the moderately difficult case (case V), which had a lower concentration of OCBs.

Subgroups within both the EPS and IEF groups were analyzed to compare the performance of specific combinations of methods (see Table 1). EPS was most frequently combined with gold staining after blotting to PVDF membranes, whereas IEF was mostly combined with immunoblotting (Table 1). None of the EPS subgroups had good general performance. Almost all the correct results for cases VI and VII were achieved by the participants using gold staining after blotting. Subdivision of the IEF group was very informative. Those using IEF in combination with immunoblotting achieved a very high percentage of correct results. The relatively low score of the IEF group for cases VI and VII could be attributed almost entirely to those participants not using this method (Table 1).

From this analysis it can be concluded that, in general, all methods will provide the correct result and interpretation when OCBs are absent or for the detection of a monoclonal band in both CSF and serum. However, in line with other data *(8)*, only the combination of IEF with immunoblotting had a high sensitivity for the detection of CSF-restricted OCBs (type 2), especially when they were present in low concentrations, as well as for the detection of the so-called "mirror pattern" of OCBs (type 4). Although EPS with gold staining after blotting appears to be an attractive alternative for many laboratories, possibly guided by the better performance than EPS in combination with other detection systems, the performance of this combination of methods was inferior to that of IEF with immunoblotting. Although IEF with direct protein staining provided slightly better results than EPS with direct protein staining, the former method was also inferior to IEF with immunoblotting. In line with our conclusions, agarose gel EPS has recently been discarded as a method for the detection of OCBs *(9)*. On the basis of our data, we also do not advocate the use of immunofixation (high-resolution) EPS, which has been promoted for its simplicity *(10)*, speed, cost-effectiveness, and reproducibility *(11)*.

Two major problems, however, may occur in the interpretation of the OCB patterns obtained with IEF combined with immunoblotting. The first problem, as observed for case III, is that a monoclonal band in both the CSF and serum (type 5) may be misinterpreted as a mirror OCB pattern (type 4). However, because IEF of monoclonal IgG produces a very specific ladder pattern of four to five bands equally spaced from each other with descending intensity toward the anodic part of the blot (see Fig. 1 in the online data supplement), it is very likely that a lack of experience in the recognition of this pattern may lead to a false interpretation. The second problem is that false-positive "pseudo-banding" may occur because of discontinuous pH gradients. However, inclusion of several negative control samples in each assay will help to minimize such false-positive results.

In conclusion, we recommend IEF with immunoblotting as the most suitable method for the detection of OCBs in CSF and serum samples at present. In addition, it has been concluded that this method has a high interlaboratory reproducibility *(12)*. Although it may implicitly be known to many clinical chemists that IEF with immunoblotting is the optimal method for detection of OCBs in CSF, to our knowledge this is the first systematic analysis of the various methods that are in use. The conclusions from this analysis are in line with recommendations made by both laboratory and clinical specialists *(1, 3, 6)*. Application of other methods, although they may be rapid, simple, and inexpensive, may lead to misdiagnosis of neurologic disease and delay therapeutic intervention for patients and should be replaced by analysis by a combination of IEF and immunoblotting.

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Comparable Effects of DIGIBIND and DigiFab in Thirteen Digoxin Immunoassays, *Gwendolyn A. McMillin,[1] William E. Owen,[2] Thomas L. Lambert,[3] Barun K. De,[4] Elizabeth L. Frank,[1] Phillip R. Bach,[5] Thomas M. Annesley,[6] and William L. Roberts[1*]* ([1] Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT 84132; [2] ARUP Institute for Experimental and Clinical Pathology, Salt Lake City, UT 84108; [3] Reno Veterans Affairs Medical Center, Reno, NV 89520; [4] University of Mississippi Medical Center, Jackson, MS 39216; [5] Primary Children's Medical Center, Salt Lake City, UT 84113; [6] University of Michigan, Ann Arbor, MI 48109; * address correspondence to this author at: c/o ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108-1221; fax 801-584-5207, e-mail william.roberts@aruplab.com)

Digoxin is widely prescribed for the treatment of cardiac conditions *(1)*. Because of its narrow therapeutic range, digoxin-related toxicity resulting from acute or chronic overdose is common. Metabolites of digoxin as well as related compounds, including digitoxin, tanshinones, bufandienolide, and oleander, can contribute to or independently produce digoxin toxicity *(2, 3)*. Digoxin toxicity can be rapidly and safely reversed by administration of anti-digoxin immune fragments (Fab) such as DIGIBIND®, which has been available in the US since 1986. Therapeutic Fab products act by binding digoxin with high affinity (10^9–10^{10} L/mol), favoring movement of digoxin out of tissue and thus promoting elimination. Factors that impact dosing with Fab products include known or suspected digoxin load, patient weight and history, and renal function *(4–7)*.

Monitoring the free digoxin concentration after Fab administration may help ensure appropriate dosing, prevent deadly recrudescent toxicity, and determine when digoxin therapy should be resumed *(8–10)*. Monitoring free digoxin in serum is challenged by the positive interference that has been extensively described with DIGIBIND, which interferes with immunoassays by competing with assay capture antibodies. The degree of interference depends on incubation times, washing steps, and the affinity of capture antibody for bound vs free digoxin *(11–13)*. Consequently, monitoring of free digoxin in ultrafiltrates is a popular strategy for managing DIGIBIND-treated patients. Although ultrafiltration eliminates interference produced by large molecules, such as endogenous digoxin-like immunoreactive factors (DLIFs) and DIGIBIND, it does not eliminate interferences produced by small molecules known to interfere with digoxin immunoassays, such as spironolactone *(14)*. In addition, ultrafiltration methods are not standardized, may require matrix-specific calibration, add expense and manual manipulation, and lengthen turnaround time *(8, 15, 16)*.

Fig. 1. Plots illustrating in vitro interference of DIGIBIND or DigiFab with 13 digoxin immunoassays.

Group A (*panels A1–A4*) includes methods with marked interference; group B (*panels B1–B4*) includes methods with moderate interference; and group C (*panels C1* and *C2*) includes methods with minimal interference. Within each group, *panels 1* or *3* represent assays performed on samples treated with DIGIBIND, *panels 2* or *4* represent assays performed on samples treated with DigiFab. Within groups A and B, *panels 1* or *2* represent assays in the absence of digoxin, *panels 3* or *4* represent assays in the presence of 40 μg/L digoxin. In group C (*panels C1* and *C2*), only data obtained in the presence of 40 μg/L digoxin are shown with ultrafiltrates (*UF*) for the TDx and AxSYM assays.

DigiFab™ is a Fab preparation that was approved by the Food and Drug Administration in 2001 for treating potentially life-threatening digoxin toxicity or overdose. Fab is produced by immunization of sheep with digoxin (DIGIBIND) or digoxindicarboxymethylamine (DigiFab), followed by purification of the Fab from blood. The approximate molecular weights of DigiFab (46 000) and DIGIBIND (46 200) are similar, and a single vial of either DIGIBIND or DigiFab will bind ~0.5 mg of digoxin in vivo. As such, the clinical claims, dosing recommendations, and administration of DigiFab are identical to those of DIGIBIND. However, clinical studies have monitored DigiFab therapy by measuring digoxin in ultrafiltrates only *(17)*. The present study was designed to determine whether clinically relevant concentrations of DigiFab in serum interfere with 13 digoxin immunoassays and to compare results with DIGIBIND.

Single vials of DIGIBIND (38 mg; Glaxo Wellcome Inc.) and DigiFab (40 mg; Protherics, Inc.) were dissolved in 4 mL of type 1 water. DIGIBIND and DigiFab were added to pooled drug-free and DLIF-free serum to obtain final concentrations of 0.1, 0.8, 2, 8, and 16 mg/L. Increasing concentrations of each antidote (1, 2, 4, 8, and 16 mg/L) were also combined with 40 μg/L digoxin (Sigma-Aldrich) in pooled serum. Ultrafiltrates were prepared by use of Millipore Centrifree filters (30 kDa) and a fixed-angle centrifuge rotor (2000*g* for 30 min at ambient temperature). Prepared samples were aliquoted and stored frozen (−70 °C) until analyzed, a practice that should not affect the ability of DIGIBIND to bind digoxin *(18)*.

Digoxin was measured by 13 commercially available competitive immunoassays. Five homogeneous assays (Beckman-Coulter Synchron; Roche CEDIA, Integra, and TinaQuant; and Syva Emit 2000) and seven heterogeneous assays (Abbott AxSYM, Beckman-Coulter Access, Chiron ADVIA Centaur, Dade Behring Dimension RxL, DPC IMMULITE 2000, Ortho Vitros, and Roche Elecsys 2010) designed to quantify free digoxin in patient serum or plasma were evaluated. The CEDIA and TinaQuant as-