The Western Immunoblot for Lyme Disease: Determination of Sensitivity, Specificity, and Interpretive Criteria with Use of Commercially Available Performance Panels

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Recent recommendations for the serological diagnosis of Lyme disease include statements on quality assurance and the use of performance panels to assess laboratory competency. We used two performance panels—one from the Centers for Disease Control and Prevention (CDC) and one from Boston Biomedica Inc. (West Bridgewater, MA)—to evaluate the sensitivity and specificity of four western blot kits. We used the same panels to compare the interpretive criteria for western blots as proposed by participants in the Centers for Disease Control and Prevention, Association of State and Territorial Public Health Laboratory Directors Conference and those proposed by BBI Clinical Laboratories (BBICL; New Britain, CT). Our results indicated that the BBICL western blots were more sensitive than those of the CDC, MarDx (Carlsbad, CA), or Cambridge Biotech (Rockville, MD). However, use of the CDC criteria with the BBICL western blots increased specificity to 100% but reduced sensitivity to 74.3%. A sample table is provided as an example of the test results obtained with the BBI performance panel. Obviously, this work should be confirmed by other investigators.

In response to numerous reports on problems associated with Lyme disease testing [1–3], participants in the recent Centers for Disease Control and Prevention (CDC), Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) Conference on the Serological Diagnosis of Lyme Disease [4] made several recommendations including:

(1) Lyme disease testing should be performed only in laboratories that have comprehensive quality assurance programs.

(2) Serum samples used to evaluate screening tests or western blots in proficiency testing should cover all stages of Lyme disease, and samples should be representative of the target population. Each sample should be from a single donor.

(3) A repository of serum specimens from patients with well-characterized *Borrelia burgdorferi* infections (early and late), other spirochetal infections, other infections and inflammatory disorders that have shown cross-reactivity in Lyme disease testing, and normal serum samples from areas of nonendemicity should be maintained by the CDC. Industry should provide resources to develop appropriate serum panels. These panels should be made available to research and development laboratories and to testing laboratories for validation studies. At least two such panels are currently available: one, which comprises a 45–47-member panel, is available from the CDC, and the other, which comprises a 15-member mixed titer panel, is available from Boston Biomedica (West Bridgewater, MA).

Materials and Methods

The CDC performance panel was used to evaluate the sensitivity and specificity of three western blot products (BBI Clinical Laboratories [BBICL; New Britain, CT], MarDx [Carlsbad, CA], and Cambridge Biotech [Rockville, MD]). In a separate evaluation, the CDC panel was also used to compare the BBICL western blot and the CDC western blot. The Boston Biomedica Lyme Disease Mixed Titer Performance Panel can also be used to validate new Lyme disease antibody tests and to compare the sensitivity and specificity of a newly adopted antibody test.

Each of the serum samples in the CDC panel has limited clinical classification, including presence/absence of erythema migrans (EM), culture results, and whether the patient was IgG/IgM reactive or seronegative. The western blot and ELISA results on this panel are not available to the purchaser until the testing has been performed and sent to the CDC for analysis; only then are the reference results released. Hence, use of the panel is blinded. While clinical characterization is provided, there are no data available on when the specimens were collected in reference to the appearance of EM or a culture positive for *B. burgdorferi*.

We compared three western blot kits for the detection of IgM and IgG antibodies to *B. burgdorferi*. They included the BBICL western blot kit, made by Biotech Research Laboratories (BBI), the MarDx kit, and the Cambridge Biotech kit.

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MarDx IgM Western Blot
BBI

Panel ID #	23	39	41	Result*	30	31	34	37	58	66
	-Significant IgM Bands-				----Other IgM Bands----					
PTL202-01	23		41	POS						
PTL202-02	23		41	POS						
PTL202-03†		39	41	POS	30		34	37		
PTL202-04†				NEG						
PTL202-05	23		41	POS		31			58	66
PTL202-06				NEG						
PTL202-07	23		41	POS						
PTL202-08				NEG						
PTL202-09				NEG						
PTL202-10	23	39		POS			34			
PTL202-11				NEG						
PTL202-12				NEG						
PTL202-13				NEG						
PTL202-14	23		41	POS						
PTL202-15	23			NEG		31				
Run Date:	11/25/95									
Kit Lot #:	6098									
Exp. Date:	8/96									
Product #:	40-265M									

*MarDx IgM Western Blot Interpretation:
POS = Positive = 2 or more significant bands
NEG = Negative = Less than 2 significant bands

MarDx IgG Western Blot
BBI

18	23	28	30	39	41	45	58	66	93	Result**	31	34	37	60
											-Other IgG Bands-			
	-Significant IgG Bands-													
18	23				41					NEG				
18			30	39	41	45	58	66	93	POS				60
18			30	39		45	58		93	POS				60
18			30	39		45	58		93	POS				60
	23	28								NEG		34		60
18	23	28	30	39		45	58		93	POS				60
	23				41	45				NEG				
										NEG	31			
										NEG				
18	23		30	39	41		58		93	POS				60
18	23	28	30	39	41	45	58	66	93	POS			37	60
18	23	28	30	39		45	58		93	POS				60
18	23	28	30	39	41		58		93	POS				60
18	23				41					NEG				
18	23		30	39	41		58	66	93	POS	31	34		60
11/24/95														
6086														
8/96														
40-266SG														

**MarDx IgG Western Blot Interpretation:
POS = Positive = 5 or more significant bands
NEG = Negative = Less than 5 significant bands

†Member #03 and 04 are from the same donor; member #04 was drawn approximately 5 months after member #03.

Figure 3. A representative sample of results for panel members of a BBI Performance Panel for a MarDx (Carlsbad, CA) western blot kit.

considered reactive. Results with use of indirect fluorescent antibody are endpoint dilutions.

There are no universally accepted criteria for western blot interpretation; therefore, the interpretation of the band pattern was based on the manufacturers' criteria for their kits and the in-house criteria (BBICL) for the in-house methods. Figure 3 shows a representative sample of the results provided with the panel, in this case western blot results for panel members of a MarDx western blot kit. This performance panel will be invaluable to both kit manufacturers and hospital laboratory personnel who wish to validate their diagnostic procedures for Lyme disease. While this Lyme disease panel is antibody based, PCR is becoming more widely used for the laboratory diagnosis of Lyme disease [5]. Molecular panels are now needed for the diagnosis of Lyme disease.

Conclusion

The results of our comparative testing of available western blot kits with use of a CDC performance panel indicated that BBICL western blots were more sensitive than those of competing manufacturers. However, application of the CDC/ASTPHLD interpretive criteria to the BBICL results increased specificity but reduced sensitivity. Sample data are also provided from a commercially available Lyme disease antibody performance panel. Use of such a panel should enable laboratory personnel to compare results with their currently used test kits to those obtained with a wide variety of kits and methods.

References

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