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

Richard C. Tilton, Mary N. Sand, and Mark Manak

From BBI Clinical Laboratories, Inc., New Britain, Connecticut; and Biotech Research Laboratory, Rockville, Maryland

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 BBICL 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 46–47-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 (BBICL 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.
Comparison of three western blot kits for detection of IgM antibody to *Borrelia burgdorferi* with use of the performance panel of the Centers for Disease Control and Prevention (CDC).

<table>
<thead>
<tr>
<th>Result</th>
<th>BBICL</th>
<th>MarDx</th>
<th>Cambridge Biotech</th>
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<tr>
<td>Sensitivity (%)</td>
<td>90.0</td>
<td>78.9</td>
<td>64.3</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>86.4</td>
<td>100</td>
<td>68.2</td>
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NOTE. BBICL = BBI Clinical Laboratories (New Britain, CT).

Each assay was performed by using the procedure described in the manufacturer's product literature.

The BBICL criteria are:

- **IgM-significant bands**—23, 39, 41, 83 kD*
  - Reactive—two of the following four bands (23, 39, 41, 83 kD) must be present.
  - Equivocal—one of the following bands (23, 31, 34, 37, 39, 41, 83 kD) must be present.
  - Nonreactive—no Lyme-specific bands are present.

- **IgG-significant bands**—20, 23, 31, 34, 35, 39, 83 kD
  - Reactive—three of the following bands (20, 23, 31, 34, 35, 39, 83 kD) must be present.
  - Equivocal—one or two of the following bands (20, 23, 31, 34, 35, 39, 83 kD) must be present.
  - Nonreactive—no Lyme-specific bands are present.

The CDC/ASTPHLD criteria are:

- **IgM-significant bands**
  - Reactive—two of the following three bands (23, 39, 41 kD) must be present.
  - Nonreactive—fewer than two bands are present.

- **IgG-significant bands**
  - Reactive—five of the following bands (18, 21, 28, 30, 41, 45, 58, 66, 93 kD) must be present.
  - Nonreactive—fewer than five bands are present.

*The 83 and 93 kD bands are equivalent.*

Interpretive criteria for the BBICL western blot included both the BBICL criteria and the CDC/ASTPHLD criteria [4]. The results obtained with use of the other two kits were interpreted on the basis of the CDC/ASTPHLD criteria. Each blot was read independently by two technologists and then validated by a director, all of whom were employees of BBICL. Both the technologists and the director were blinded as to the blot manufacturer, and the specimens were coded.

Results and Discussion

Table 1 shows the results of our comparison of the three western blot products for detecting IgM, based on the CDC/ASTPHLD performance panel criteria. The BBICL IgM western blot is more sensitive than either the MarDx or Cambridge Biotech IgM western blot and slightly less specific than the MarDx IgM western blot. The BBICL criteria for IgM western blot are virtually identical to the proposed CDC/ASTPHLD criteria, except for the inclusion of the 83/93-kD band and an indeterminate category. Thus, differences in performance of the kits are probably product related and not due to differences in interpretive criteria.

Table 2 shows the results of our comparison of the three western blot products for detecting IgG with use of the CDC performance panel. On the basis of the CDC/ASTPHLD criteria, the BBICL IgG western blot is more sensitive than and as specific as the other two IgG western blot products. However, if the BBICL criteria are applied, the sensitivity of the BBICL IgG western blot increases to 87%, but its specificity is reduced. The reduced sensitivities of all IgM western blot kits, particularly those of MarDx and Cambridge Biotech, could well reflect the time at which the specimens were drawn for the panel. If the patients donated >1 unit of plasma months after the initial acute episode of Lyme disease, then the IgM titer would be expected to be diminished.

The second study was also done in blinded fashion at BBICL. IgG and IgM western blots were performed on the 46-member CDC panel, and the results were sent to the CDC for analysis. A comparison of results obtained with BBICL or CDC western blots and clinical information are shown in figures 1 and 2. BBICL IgM western blots classified 28 of 30 patients with Lyme disease as IgM positive (figure 1). The diagnosis was missed in two patients (6%). The CDC reported apparently false-negative IgM western blots for 10 patients, all of whom were symptomatic. Of these 10 patients who were negative by the CDC IgM western blot and positive or equivocal by BBICL IgM western blot, the one patient who was positive by the BBICL IgM western blot had confirmed EM and was culture positive for *B. burgdorferi*. Of the nine patients who were negative by the CDC western blot and equivocal by the BBICL western blot, seven were positive for IgG antibodies to *B. burgdorferi* by western blot in both laboratories, and two were negative for IgG antibodies in both laboratories. All nine patients had confirmed EM and cultures positive for *B. burgdorferi*.

BBICL reported that 35 of 46 patients had either positive (19 patients) or equivocal (16 patients) IgG western blots (figure 2). Of these 35 patients with clinically defined Lyme disease, 23 were found to be seropositive with use of the CDC western blot. There were 12 false-negative results. One of the specimens negative for IgG by the CDC western blot but positive by the BBICL western blot was from a *B. burgdorferi*–infected patient whose IgM western blot was found to be positive in both laboratories. The 12 patients who were negative by the CDC/ASTPHLD criteria but had confirmed Lyme disease were equivocal by the BBICL criteria. Seven of these *B. burgdorferi*–infected patients had a positive IgM western blot in both laboratories.

Four patients had a history of tick bite, EM, and positive cultures. There were no clinical data for one patient. There were five patients who were positive by the CDC/ASTPHLD criteria and equivocal by the BBICL criteria. There were multi-
ple bands on both western blots for all five of these patients, but there were not enough bands to fulfill the BBICL criteria for positivity. Thus, BBICL classified all (35) patients who met the CDC criteria for Lyme disease as either positive or equivocal, while the CDC western blot results indicated that 12 patients who met the CDC criteria for Lyme disease were negative. The case of one patient who was western blot–positive for IgM at the CDC and negative for IgM with use of the BBICL western blot is still unresolved. In addition, five specimens were equivocal with use of the BBICL IgG western blot but positive with use of the CDC IgG western blot.

The BBICL western blot appears to be more sensitive than the CDC western blot, the MarDx western blot, or the Cambridge Biotech western blot. Of course, the issue is whether a western blot should be more sensitive than specific, or vice versa. If the western blot is to be used solely for confirmation of the results of ELISA, then specificity may be more desirable than sensitivity. However, a specific western blot with low sensitivity may invalidate a sensitive and specific ELISA. A highly sensitive and specific western blot is desirable for a two-tiered test system. Of major significance is the fact that despite the CDC recommendation for two-tiered testing, many physicians who treat patients with Lyme disease do not believe that an ELISA is an appropriate screening test and consequently request that both ELISA and western blot be done.

If the western blot is to be used in this manner, then sensitivity may be preferred over specificity, particularly for patients with acute Lyme disease or for those with suspected persistent disease and symptoms that are not commonly observed. We recognize, however, that reduced specificity may further complicate the serodiagnosis of Lyme disease because of the potential for increased numbers of false-positive tests.

Workers at Boston Biomedica have assembled a set of 15 aliquots of frozen serum and plasma units with reactivity to *B. burgdorferi* ranging from negative to strongly positive when used with a variety of currently available test methodologies. Samples have been selected to demonstrate IgG and/or IgM reactivity. In addition, one negative plasma unit has been included as a nonreactive control. These specimens are undiluted aliquots from plasma and serum units collected from 1994 to 1995. The units were processed by sterile filtration. No preservatives were added. Clinical information on panel members was included when available. The purpose of this performance panel of naturally occurring serum and plasma samples is to enable manufacturers and diagnostic laboratories to evaluate their tests for detection of antibodies to *B. burgdorferi* with characterized samples and to provide comprehensive data for comparative analysis.

The tables provided in the Panel give results from both commercially available test kits and in-house procedures performed at BBICL, Boston Biomedica, and internationally recognized reference laboratories. Product numbers are indicated for identification of each method. Numeric results are the means of duplicate tests. Some results are expressed as signal-to-cutoff ratios to facilitate comparisons among kits; ratios of $\geq 1.0$ are

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<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>87.2</td>
<td>74.3</td>
<td>47.0</td>
<td>43.6</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>60.0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
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Figure 1. Comparison of the sensitivity and specificity of BBI Clinical Laboratory (New Britain, CT) and Centers for Disease Control and Prevention western blots for IgM antibody to *Borrelia burgdorferi* with that of clinical information (culture results, the presence of erythema migrans [EM], and serology) in the detection of *B. burgdorferi* infection.

Figure 2. Comparison of the sensitivity and specificity of BBI Clinical Laboratory (New Britain, CT) and Centers for Disease Control and Prevention western blots for IgG antibody to *Borrelia burgdorferi* with that of clinical information (culture results, the presence of erythema migrans [EM], and serology) in the detection of *B. burgdorferi* infection.
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