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Comparison of Western Immunoblotting and the C6 Lyme Antibody Test for Laboratory Detection of Lyme Disease

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Three commercial Lyme disease Western immunoblotting (WB) kits and the C6 *Borrelia burgdorferi* (Lyme) enzyme-linked immunosorbent assay (ELISA) kit were compared using two commercially available performance panels from the Centers for Disease Control and Prevention (CDC) and Boston Biomedica (BBI). Combined, the panels consisted of 52 characterized specimens. Immunoglobulin G (IgG) sensitivity was similar for the three WB products. The BBI and Marblot WBs were more specific for IgG antibodies, while the Virablot was the most sensitive for IgM antibody. The BBI WB was 100% specific for IgM, while Marblot was 97% and Virablot was 77% specific for IgM. The C6 ELISA was found to be 100% sensitive. Four false-positive C6 results were identified in patients that had clinically and microbiologically confirmed Lyme disease but were not detected by the CDC reference methods. No one WB product showed overall superiority. The C6 ELISA shows promise as the first ELISA for Lyme disease that would not require a supplemental test such as a WB.

Early tests for the serological detection of *Borrelia burgdorferi* antibodies in patients suspected of having Lyme disease (LD) lacked both sensitivity and specificity (1, 5). In 1989, Fister et al. (4) reported on the available serological tests for LD and suggested that all positive tests be confirmed by Western immunoblotting (WB). In 1995, the Dearborn Conference held by the Centers for Disease Control and prevention (CDC) and the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) recommended that all indeterminate and positive enzyme-linked immunosorbent assays (ELISAs) be confirmed by WB (2). Tilton et al. (11) evaluated the available WB kits by using commercially available performance panels and reported differences in specificities and sensitivities. That study and the present one were, in part, a response to the recommendations of the CDC-ASTPHLD Dearborn Conference participants, who stated that (i) LD testing should only be performed in laboratories with comprehensive quality control systems, (ii) serum samples used to evaluate diagnostic products should cover all stages of LD, and (iii) a repository of characterized specimens should be available for comparative testing of diagnostic products for LD (2).

There have been no reports on the performance of confirmatory serological tests for LD since 1998. Tilton recently reviewed new serological tests for LD (10), and although substantial progress toward the sensitive and specific detection of immunoglobulin G (IgG) and IgM antibodies to *B. burgdorferi* has been made, there is presently no definitive evidence that any of these tests are capable of being stand-alone tests with no confirmatory or supplemental WB. Some of these tests, such as the C6 Lyme antibody test (8) and the VlsE antibody test (6), show promise because of their high specificity and acceptable sensitivity in all stages of the disease.

As of early 2004, three Lyme WB products were available in the United States: the Marblot (MarDx, Carlsbad, Calif.), the Boston Biomedica (BBI; West Bridgewater, Mass.) WB test kit, and the Virablot (Viramed, Steinkirchen, Germany). Two other products, QualiCode (Immunetics, Cambridge, Mass.) and a WB kit from Focus Technologies (Cypress, Calif.) were unavailable because of reformatting and withdrawal from the market, respectively. Of the three available products, two (Marblot and the BBI kit) are FDA approved. The Virablot kit is for research purposes only pending FDA review and approval.

This study used two performance panels containing a total of 57 characterized serum and plasma specimens. One panel is from the CDC and consists of 42 samples, and the other is from BBI and includes 15 samples. These performance panels were used to evaluate the sensitivities, specificities, and operating characteristics of the Marblot, the BBI WB, the Virablot, and the C6 Lyme antibody test.

MATERIALS AND METHODS

Performance panels. The CDC LD evaluation panel is commercially available and consists of 42 characterized serum samples, both positive and negative. Each of the serum samples was provided with limited clinical information on the presence or absence of erythema migrans (EM), culture results, if available, and whether the patient was IgG and/or IgM seroreactive. Specimen collection times are also now included with this panel, unlike when the panel was tested in 1997 (11). Similarly, reference IgG and IgM WB results are provided in the panel insert, unlike in 1997 when the panel was tested blindly and reference results were released only upon receipt of experimental results. The CDC reference WB results were generated with the Marblot that was used to confirm a MarDx ELISA.

The BBI mixed-titer performance panel, catalog no. PTL202, is also available commercially. The purpose of the panel is to enable manufacturers and diagnostic laboratories to validate their kits by using well-characterized serum samples. The panel consists of 11 positive plasma specimens, 3 positive serum specimens, and 1 negative plasma specimen. The panel provided clinical information confirming the diagnosis of LD for seven of the panel members. The remaining seven positive specimens and the single negative plasma sample had no accompanying clinical information. None of the panel members had information on the time of specimen collection relative to the onset of symptoms.

The differences between the two panels were that there was less clinical information with the BBI panel and, unlike the CDC panel, the BBI panel provided test results using 11 commercially available LD ELISA kits, 3 WB kits (Marblot, an in-house WB, and a GenBio kit), and results from internationally

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TABLE 1. CDC-ASTPHLD Dearborn interpretation criteria for LD WBs

WB antibody	Result	CDC interpretation criteria[a]
IgM	Negative (nonreactive)	Fewer than 2 bands must be present (23, 39, 41 kDa)
	Positive (reactive)	Two or more bands must be present (23, 39, 41 kDa)
IgG	Negative (nonreactive)	Fewer than 5 bands must be present (18, 23, 28, 30, 39, 41, 45, 58, 66, 83 to 93 kDa)
	Positive (reactive)	Five or more bands must be present (18, 23, 28, 30, 39, 41, 45, 58, 66, 83 to 93 kDa)

[a] See reference 2.

known reference laboratories using research-level testing. The GenBio dot blot contains four Lyme-specific antigens: OspC, p39, flagellin, and a high-molecular-mass protein (83 to 93 kDa). The specimens from both sources were undiluted, and no preservatives were added. The BBI panel members were collected between 1994 and 1995, and aliquots have been frozen at −80°C since collection. The Marblot and Virablot WB kits were purchased from the manufacturers, and the BBI WB kits were provided to Medical Diagnostic Laboratories by BBI for evaluation.

BBI Lyme WB test kit. The BBI WB kit uses sodium dodecyl sulfate-solubilized *B. burgdorferi* strain 2591 proteins that are separated by gel electrophoresis and transferred to a nitrocellulose membrane. Strain 2591 is one of the three *B. burgdorferi* strains recommended by the CDC-ASTPHLD report (2). The membranes are processed according to the product insert. Positive and negative controls are included. Strips are developed, and the reaction is terminated after 10 to 12 min of incubation. Bands are identified using an IgG and IgM blot reading guide consisting of a previously developed and mounted strip from the same strip lot by BBI. One band on the reading guide is identified as the minimum intensity (threshold) band. Only bands of intensity equal to or greater than this minimum are scored. Blots are interpreted using the CDC criteria (2) (Table 1).

MarDx *B. burgdorferi* Marblot strip test system. The Marblot product insert indicates that the B31 strain of *B. burgdorferi* was used. Each kit with either the IgG or the IgM test includes both reactive and negative controls. Reactive controls must include all significant bands, while the negative controls show no significant bands. The WB strips are processed according to the product insert and are typically developed for 4 to 12 min. The reaction is terminated when the bands on the weakly reactive control become slightly visible. A serum band locator sample is included for processing with each WB run, as is a weakly reactive control and a blot banding template. When reading the blots, the blot banding template is used to locate the bands on the serum band locator, which is, in turn, used to read the test strips. The 41-kDa band on the weakly reactive control is used as the intensity standard to which each band on a test strip is compared. Bands weaker than the intensity standard are not scored, even if present and visible. Only those bands indicated as being significant in the CDC criteria for IgG or IgM antibodies are used for interpretations of positive or negative (2).

Viramed *Borrelia* Virablot test kit. The Virablot WB test kit uses proteins from two European isolates: *B. burgdorferi* sensu stricto, which is analogous to the American isolate B31, and *Borrelia afzelii*, a component of the *B. burgdorferi* sensu lato complex. According to the product insert, this antigen combination guarantees optimum sensitivity and specificity of the assay. Each test strip has an integrated conjugate reactivity control which indicates whether the test is for IgG or IgM as well as a functional control which, when positive, indicates that all reagents were added. Each kit includes a developed positive control strip that is used as a band locator. The Virablot also uses IgG and IgM cutoff controls. Incubation is stopped when the IgG 41-kDa band or the IgM 23-kDa band becomes clearly visible. Bands are scored as weak (intensity < cutoff), clear (intensity = cutoff), strong (intensity > cutoff), or extremely strong (color of the 41-kDa band is violet to black). For use in the United States, the band patterns are interpreted using the CDC criteria and reported as positive or negative (2) (Table 1).

Procedure. Each serum sample from the BBI mixed-titer performance panel was repeated on two separate occasions for each WB kit evaluated. The CDC samples were not tested in duplicate due to limitations imposed by the provided sample volumes. All tests were performed according to instructions provided by the respective manufacturers, with the exception that an automated WB processor (Autoblot 2000; MedTec Inc., Chapel Hill, N.C.) was used for all runs. Each strip was read blindly by two technologists, and the results were independently recorded before review by a senior scientist. Quality control procedures included those specified by the manufacturer plus the inclusion of both positive and negative controls in each WB run. Bands were scored as present or absent using the intensity standard described in the product insert from each manufacturer.

C6 *B. burgdorferi* (Lyme) ELISA kit. The C6 ELISA was performed in duplicate on all members of both panels according to the manufacturer's (Immunetics) instructions. Results were scored as negative (ELISA index score, ≤0.90), equivocal (0.91 to 1.09), or positive (≥1.1).

RESULTS

Table 2 summarizes the available clinical information with respect to EM and culture positivity for *B. burgdorferi* that was presented with the CDC performance panel. The BBI performance panel also indicated that 4 of 15 specimens were positive for EM: 202-01-BBI, 202-07-BBI, 202-10-BBI, and 202-14-BBI.

Table 3 shows the results for each specimen that were obtained by testing the two performance panels with the three WB kits and the C6 ELISA. Scoring of the WB strips to

TABLE 2. Erythema migrans and *B. burgdorferi* culture status of CDC specimens

Specimen(s)	Erythema migrans	Culture
90-0874, 90-0875, 90-0876, 90-0877, 90-0878, 90-2111, 90-2436, 90-2668, 91-0531, 91-0532, 91-0544, 91-0794, 91-0943	Not reported	Not performed
90-2631, 91-1222, 91-1347, 91-1348, 91-1349, 91-1350, 91-1351, 91-1352, 91-1353, 91-1354, 91-1458, 91-1841, 91-1842, 91-1843, 91-1844, 91-1845, 91-1846, 91-1847, 92-1682[a]	Positive	Positive
91-0521, 91-0533, 91-0865, 91-1104	Positive	Not performed
91-0900[b]	Not reported	Negative
92-0057[c]	Not reported	Positive
92-1941, 93-0208, 93-1414, 93-1426[a]	Indeterminate	Positive

[a] The indicated specimens were obtained by skin punch biopsy.
[b] The indicated specimen was obtained from joint fluid.
[c] The indicated specimen was obtained from cerebrospinal fluid.

TABLE 3. BBI and CDC performance panel results for three LD WB kits and the C6 ELISA[a]

Specimen(s)	IgG				IgM				IgG/IgM C6
	RR	MB	VM	BBI	RR	MB	VM	BBI	
BBI panel[a]									
202-08, 202-09	−	−	−	−	−	−	−	−	−
202-01	−	−	−	−	+	−	+	+	+
202-05, 202-07, 202-14	−	−	−	−	+	+	+	+	+
202-04	+	−	−	−	−	−	−	−	+
202-06, 202-11, 202-12, 202-13	+	+	+	+	−	−	−	−	+
202-15	+	+	+	+	−	+	+	−	+
202-02	+	+	+	+	+	−	−	−	+
202-03	+	+	+	+	+	−	+	−	+
202-10	+	+	+	+	+	−	+	+	+
CDC panel[b]									
90-0875, 90-0876, 90-0877, 90-2631, 91-1458, 93-0208	−	−	−	−	−	−	−	−	−
90-0874, 90-0878, 91-1845	−	−	−	−	−	−	+	−	−
91-1352, 91-1846, 92-1682	−	−	−	−	−	−	−	−	+
91-1354	−	−	−	−	−	−	+	−	+
91-1350, 91-1843	−	−	−	−	+	−	+	+	+
91-1104, 91-1347, 91-1349, 92-1941, 91-1841, 91-1847	−	−	−	−	+	+	+	+	+
91-1351	−	−	+	−	+	−	+	+	+
91-1353	−	−	+	−	+	+	+	+	+
91-1222	−	+	+	+	+	+	+	+	+
91-0521	+	+	−	+	−	−	−	−	+
91-1842	+	−	−	−	−	−	−	−	+
93-1426	+	−	+	−	−	−	+	−	+
93-1414	+	−	−	−	+	+	+	+	+
91-0544	+	+	+	+	+	−	−	−	+
91-0865, 92-0057	+	+	+	+	+	+	−	−	+
90-2111, 91-0794, 91-0531, 91-0532, 91-0900, 91-0943	+	+	+	+	−	−	−	−	+
90-2436	+	+	+	+	−	−	+	−	+
91-1348	+	+	+	+	+	−	+	+	+
91-1844	+	+	+	+	+	−	+	+	+
90-2668	+	+	+	−	+	+	+	+	+
91-0533	+	+	+	+	+	+	+	−	+

[a] Abbreviations: RR, reference result provided with panel; MB, Marblot; VM, Virablot; BBI, BBI WB; C6, C6 ELISA.
[b] For specimens in the BBI panel, the percentages of positive results were as follows: in the IgG RR, MB, VM, and BBI, 60, 53, 53, and 53%, respectively; in the IgM RR, MB, VM, and BBI, 47, 27, 47, and 33%, respectively; in the C6, 87%.
[c] For specimens in the CDC panel, the percentages of positive results were as follows: in the IgG RR, MB, VM, and BBI, 43, 38, 43, and 36%, respectively; in the IgM RR, MB, VM, and BBI, 45, 31, 52, and 36%, respectively; in the C6, 79%.

achieve a negative or positive IgM or IgG result was based upon the CDC-ASTPHLD criteria shown in Table 1.

Tables 4 and 5 show the comparative performances of the three WB kits and the C6 ELISA both for the BBI and CDC panels combined and for each individual panel. When the BBI panel results for IgG antibodies alone were examined, it was found that there was no significant difference in the sensitivity and specificity values between the three commercial kits. The sensitivity ranged from 89 to 90%, and the specificity was 100%. The overall agreement for IgG with the consensus reference methods as indicated in the BBI panel brochure was 93%. The IgM sensitivity results were not consistent, ranging from 43% for Marblot to 71% for the BBI kit and 86% for Virablot. The IgM specificity ranged from 88% for Marblot and Virablot to 100% for the BBI WB kit.

When the three WB kits were used to test the 42-member CDC performance panel, it was found that the IgG sensitivity was 83% (Marblot and Virablot) and 78% (BBI WB), while the IgG specificity was 96% (Marblot and BBI WB) and 88% (Virablot). The IgM sensitivities were 68% (Marblot), 84% (Virablot), and 79% (BBI WB). The IgM specificities were 74% (Virablot) and 100% (BBI and Marblot). The overall agreements with the CDC reference results were 90 and 86% (Marblot IgG and IgM), 86 and 79% (Virablot IgG and IgM), and 88 and 90% (BBI WB IgG and IgM).

Composite results of the three WB kits tested against the 57 combined BBI and CDC specimens showed that the IgG sensitivity was 85% for both Marblot and Virablot and 81% for the BBI WB. The IgG specificity was 97% for both Marblot and the BBI WB and 90% for Virablot. The overall agreement with the 57 characterized specimens was 88 to 91%. The IgM sensitivites were 62% (Marblot), 85% (Virablot), and 77% (BBI WB), while the IgM specificities were 97% (Marblot), 77% (Virablot), and 100% (BBI WB). The overall agreement for IgM WB was 81% for Marblot and Virablot and 89% for the BBI WB. Tables 4 and 5 also show the results of the C6 ELISA compared to the consensus results of both the CDC and the BBI panels. The reference IgG and IgM results were combined since the C6 test detects both IgG and IgM antibodies. The sensitivity was 100%, the specificity was 73%, and the overall agreement was 93%.

There were four false-positive C6 ELISA results. All WB results for these four specimens, including the reference Marblot and the WBs being evaluated, were interpreted as negative with the exception of 91-1354-CDC, which was Virablot IgM positive. Clinical information on all four of these patient sam-

TABLE 4. Results of three LD WB tests and the C6 ELISA for the BBI and CDC performance panels

Test and result	No. of results with indicated reference result[a]:	
	Positive	Negative
IgG Marblot		
Positive	23 (8/15)	4 (1/3)
Negative	1 (0/1)	29 (6/23)
IgG Virablot		
Positive	23 (8/15)	4 (1/3)
Negative	3 (0/3)	27 (6/21)
IgG BBI WB		
Positive	22 (8/14)	5 (1/4)
Negative	1 (0/1)	29 (6/23)
C6 ELISA		
Positive	42 (13/29)[b]	0 (0/0)
Negative	4 (0/4)[c]	11 (2/9)[c]
IgM Marblot		
Positive	16 (3/13)	10 (4/6)
Negative	1 (1/0)	30 (7/23)
IgM Virablot		
Positive	22 (6/16)	4 (1/3)
Negative	7 (1/6)	24 (7/17)
IgM BBI WB		
Positive	20 (5/15)	6 (2/4)
Negative	0 (0/0)	31 (8/23)

[a] Results are positive or negative according to MarDx WB reference results provided by BBI or the CDC. Data are presented as BBI + CDC (BBI/CDC), where BBI indicates results for the BBI mixed-titer performance panel and CDC indicates results for the CDC LD evaluation panel.

[b] The result indicated is a positive Lyme WB, which is positive for either IgM or IgG or for both.

[c] The result indicated is a negative Lyme WB, which is negative for both IgG and IgM.

ples, 92-1682-CDC, 91-1846-CDC, 91-1354-CDC, and 91-1352-CDC, revealed physician-diagnosed EM, cultures positive for *B. burgdorferi*, and patients who were cured after a month or less of antibiotic therapy.

To test the possibility that specimen deterioration as a result of protracted storage affected results, similar data generated in 1997 with the same CDC panel and two similar WB products (BBI and Marblot) were compared. The BBI WB showed an IgG sensitivity of 74% in 1997 compared to 81% in 2004. Marblot was less sensitive in 1997 (47%) than in 2004 (85%). The IgG specificity of both products was 100% in 1997 and 97% in 2004. These data suggest that specimen quality may not be a factor.

TABLE 5. Sensitivity, specificity, and agreement of three LD WB tests and the C6 ELISA for the BBI and CDC performance panels[a]

Ig and test	Relative sensitivity (%)	Relative specificity (%)	Overall agreement (%)
IgG			
Marblot	85 (89/83)	97 (100/96)	91 (93/90)
Virablot	85 (89/83)	90 (100/88)	88 (93/86)
BBI WB	81 (89/78)	97 (100/96)	89 (93/88)
IgM			
Marblot	62 (43/68)	97 (88/100)	81 (67/86)
Virablot	85 (86/84)	77 (88/74)	81 (87/79)
BBI WB	77 (71/79)	100 (100/100)	89 (87/90)
IgG/IgM			
C6 ELISA	100 (100/100)	73 (100/69)	93 (100/90)

[a] Data are presented as BBI + CDC (BBI/CDC), where BBI indicates percentages for the BBI mixed-titer performance panel and CDC indicates percentages for the CDC LD evaluation panel.

DISCUSSION

A critical analysis of each WB product can be performed through the examination of the combined results for 57 specimens from both performance panels (Tables 4 and 5). The IgG WB sensitivities ranged from 81 to 85%. Of the five specimens that were scored as negative by any one or more of the WB products, three samples were missed by all three kits. Sample 93-1414-CDC (Tables 2 and 3) was from a Wisconsin patient who presented with a single skin lesion and no recollection of a tick bite or other clinical manifestations of LD. The skin lesion was cultured and found to be *B. burgdorferi* positive. The serum sample included in the panel was drawn 21 days after the onset of symptoms. The IgM WBs were strongly positive, and although there were significant bands visible on all three of the IgG WBs, these were not the five required for a positive result (2). All three WB products missed bands of 18, 45, 58, and 83 to 93 kDa that were detected originally by the Marblot reference IgG WB. Sample 91-1842-CDC was from a New York patient with physician-diagnosed EM that was culture positive for *B. burgdorferi*. The sample was drawn 111 days after the onset of symptoms. Only two significant bands were observed on each of the three IgG WB products (39 and 41 kDa), but the reference IgG Marblot performed at the time of the collection was positive with 5 out of 10 possible visible bands. Samples 202-03-BBI and 202-04-BBI were drawn from the same donor approximately 5 months apart. Initially, this patient exhibited a strong positive LD serology. The 202-04-BBI IgG reference WB results at the time of collection were strongly positive, but the three WB products were scored as negative with only two or three of the five bands required for a positive test. It is possible that, for both of these samples, some antibody reactivity may have been lost as a result of being in long-term storage for the past 10 years.

The IgG specificity of the Marblot and the BBI WB was 97%, while that of the Virablot was lower at 90%. The Virablot recorded three false-positive results compared with one each for the Marblot and the BBI WB. Sample 91-1222-CDC was reported as positive by all three WBs. The Wisconsin patient from whom this sample was drawn presented with EM that was culture positive for *B. burgdorferi*. The specimen was drawn 33 days after the onset of symptoms. It is likely that this false-positive result is a true positive and that the reference WB was falsely negative (4 of 10 significant bands present). The two other samples reported as positive by the Virablot and negative by the Marblot and the BBI WB as well as the original reference WB were from patients with physician-diagnosed EM that was reported as being culture positive. The specimens were drawn 43 and 29 days after onset, respectively. The Virablot may be more sensitive for these two specimens than the Marblot, the BBI WB, or the reference Marblot.

Some of the IgM WB products did not perform as well as those for IgG. The sensitivity ranged from 62% (Marblot) to

85% (Virablot), with that of the BBI WB in between at 77%. Of the 57 total CDC and BBI panel specimens, there were between 4 and 10 false-negative results, depending on the WB product. There were two samples (91-0544-CDC and 202-02-BBI) that were missed by all three WB products. Sample 202-02-BBI was drawn from a patient with laboratory and clinically confirmed LD and exhibited no bands with any of the WBs tested. Sample 91-0544-CDC had a 23-kDa band, but no 39- or 41-kDa bands. The Marblot exhibited the worst sensitivity (62%), consistently missing the 39- and 41-kDa bands while detecting the 23-kDa band. The CDC interpretive criteria require that two of these three bands be present for a positive result (Table 1). Sample 91-0544-CDC was from a Massachusetts patient with inflammatory arthritis of the right knee. The IgG and IgM reference WBs were strongly positive at the time. The three WB kits failed to detect either the 39- or the 41-kDa band for specimens 202-02-BBI and 91-0544-CDC.

The BBI IgM WB was 100% specific, while the Marblot and Virablot had one and seven false-positive results, respectively. However, of these seven putatively negative samples detected by the Virablot, six were in the CDC panel and were from patients with culture-positive LD. The CDC reference results were negative in all six samples because either the 23- or the 41-kDa band was not detectable. The seventh specimen, 202-15-BBI, was IgG WB positive and IgM WB negative by the reference tests; no clinical information was available.

There are minor technical differences in the three WBs. The Virablot is a smaller strip, approximately one-half the width of the other two. The bands were more distinct than those of the Marblot and easier to read, even though the strip was smaller. The BBI WB strip was the same size as that of the Marblot and usually produced crisp, easily read WBs. Occasionally, the BBI WB strips had a dark speckling pattern that was not observed on the other strips. The strip development time was more difficult to control with the Marblot and the Virablot than with the BBI WB, which was robust and easily reproducible with a development time of 10 to 12 min independent of the necessity to score a control as a function of the length of incubation time. A disadvantage of the BBI WB is the total incubation time, which is 205 min compared to 165 min for the other two products.

It is possible that the C6 ELISA is a more sensitive antibody test than a WB. Liang et al. (8) and Lawrenz et al. (6) reported that the early C6 antibody response produces both IgG and IgM isotypes and may appear very soon after a tick bite. Magnarelli et al. (9) compared 11 recombinant antigens in an ELISA format and determined that VlsE was the most suitable antigen for the laboratory diagnosis of LD. While VlsE and C6 ELISAs are not identical, they vary primarily in the nature of the capture antigen; VlsE is a recombinant protein (6), and C6 is a synthetic peptide which is a component of the VlsE envelope antigen (8). There are several advantages to the use of C6 antibody tests, including no interference in patients who have been vaccinated with the 31-kDa antigen (3), detection of antibodies to the European strains of *B. burgdorferi* (7), high specificity, and a shorter reaction time (approximately 1 h). Patients with over 12 different diseases such as systemic lupus erythematosus, rheumatoid arthritis, and other spirochetal diseases were uniformly negative by the C6 ELISA (6, 8). The high sensitivity and specificity of the C6 test makes it an attractive candidate for a primary test for LD, which may obviate the need for WB confirmation.

The results can be summarized as follows: (i) the IgG WB sensitivity and overall agreement among the three kits were similar, (ii) the BBI WB and the Marblot were more specific for IgG, (iii) the IgM sensitivity was highest for the Virablot (84%) and lowest for the Marblot (62%), (iv) the IgM WB specificity was 100% for the BBI WB and 77% for the Marblot and the Virablot, and (v) the C6 antibody test was highly sensitive. Although there were four false-positive results with the C6 antibody test, all four patients had clinically and microbiologically confirmed LD with negative traditional immunological tests.

The performance characteristics of WB tests must be assessed in the context of how these serological tests are used. If the WB is used solely for confirmation of indeterminate or positive ELISA results, then specificity may be more desirable than sensitivity. However, a specific but insensitive WB may invalidate a sensitive and specific ELISA (11). For an effective two-tiered diagnostic system, a highly sensitive test and a highly specific test are desirable. Of major significance is the fact that despite the CDC recommendations, many physicians use the WB as a primary serological test or order both ELISA and WB. Unless a WB is both highly specific and sensitive, it cannot fulfill the expectations of physicians attempting to diagnose both acute and chronic or persistent LD. The answer to this enigma may be the availability of a highly sensitive and specific single test, such as the C6 antibody test.

The performance characteristics of either the WB or the C6 antibody test may be different when used on a patient population whose disease is not well documented. Particularly with LD, there is much disagreement not only on the definition of chronic disease, but also on the existence of LD in nonendemic areas of the United States. Performance panels with documented reference results are useful for test comparisons when the only "gold standard" for laboratory diagnosis is culture, a test which is technically difficult, insensitive, and not readily available.

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