Two-Tiered Antibody Testing for Lyme Disease With Use of 2 Enzyme Immunoassays, a Whole-Cell Sonicate Enzyme Immunoassay Followed by a VlsE C6 Peptide Enzyme Immunoassay

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Background. Lyme disease (LD) antibody testing currently involves a 2-tiered algorithm with a whole-cell sonicate (WCS) enzyme immunoassay (EIA), followed by IgM/IgG Western immunoblots. A single EIA using the C6 peptide of the Borrelia burgdorferi variable-major protein-like sequence expressed lipoprotein provides similar or better sensitivity but less specificity, compared with standard 2-tiered testing. Here, we investigated an alternative 2-tiered strategy, in which the first step remained a WCS EIA, but immunoblotting was replaced by a C6 EIA.

Methods. We determined the sensitivity of the 3 testing strategies with use of 91 serum samples from research study patients with LD and 78 serum samples from patients with LD whose samples were submitted to our hospital’s clinical laboratory. Specificity was measured using 54 patients with other illnesses and 1246 healthy subjects from areas where the infection is endemic and nonendemic.

Results. The 2-EIA algorithm in early LD had similar sensitivity as C6 testing alone, and both strategies had better sensitivity than did standard 2-tiered testing (61% and 64%, respectively, vs 48%; P = .03 and P = .008). For late disease, all 3 strategies had 100% sensitivity. The specificity of the 2-EIA algorithm was equal to that of standard 2-tiered testing, and both 2-tiered strategies were more specific than C6 testing alone (for both, 99.5% vs 98.4%; P = .01). The positive predictive value of the 2-EIA algorithm was 70%, compared with 66% for standard 2-tiered testing and 43% for the C6 EIA alone.

Conclusions. The 2-EIA strategy matched the individual strengths of the C6 EIA and Western blotting, without the drawbacks. The 2 EIAs provided sensitivity comparable to that of the C6 EIA but maintained the specificity of standard 2-tiered testing.
Most clinical laboratories in the United States do not attempt this procedure and, instead, rely on commercial reference laboratories, increasing both cost and turnaround time. In a recent proficiency survey administered by the College of American Pathologists, only 54 (16%) of 330 participating laboratories performed LD Western blots in-house [4].

A number of reports have shown that an EIA detecting antibodies directed against the variable-major protein-like sequence expressed (VlsE) lipoprotein of Borrelia burgdorferi or against a 26-mer peptide from the sixth invariable region of the protein performs similarly to standard 2-tiered testing [5–8]. These findings raised the possibility that the 2-tiered approach could be replaced with a single, objective, and easier to perform EIA. However, single-test approaches using a C6 EIA [9, 10], recombinant VlsE immunoassay [10], or recombinant VlsE immunoblot [11] were not as specific as the 2-tiered approach.

We recently reported a modified 2-tiered algorithm that incorporated VlsE testing [11]. The first-tier test remained a polyvalent WCS EIA, and the second-tier test was an IgG Western blot, modified by the addition of a recombinant VlsE band. IgM testing was not performed in the second tier. A key finding was that none of 252 control serum samples was positive by both WCS EIA and the VlsE band, whereas either test alone produced false-positive results [11]. Because VlsE expression in B. burgdorferi is lost or reduced after repeated in vitro passage [12, 13], spirochetal lysates used to manufacture WCS EIAs contain little VlsE, which probably explains this finding. The same study also showed that a 2-tiered algorithm consisting only of WCS EIA followed by VlsE testing on an IgG immunoblot provided sensitivity comparable to that of standard 2-tiered testing [11]. Although this modified algorithm removed one of the main drawbacks of standard 2-tiered testing (the IgM Western blot), the second-tier test (VlsE band) still relied on immunoblotting.

In response to correspondence [14], Bacon et al [15] reanalyzed data from a previous study and showed that a polyvalent WCS EIA followed by C6 IgG and pepC10 IgM ELISAs would have performed comparably to standard 2-tiered testing. Jansson et al [16, 17] designed a similar strategy for use in Europe that involved 2 IgG EIAs, one with multiple recombinant antigens and one with the C6 peptide. This approach in patients with stage 2 or 3 LD showed comparable sensitivity and better specificity than 2-tiered testing using European IgG EIA and immunoblot assays.

In the present study, we evaluated a 2-EIA strategy for LD in the United States that uses a polyvalent WCS EIA followed by a C6 EIA, for those with a positive or equivocal first-tier result. Both EIAs have been cleared by the US Food and Drug Administration (FDA) and are used widely in the United States but are not typically combined in a 2-test approach.

PATIENTS AND METHODS

Patient Samples
During phase 1, the 2-EIA algorithm was evaluated using serum samples from well-characterized patients with LD or symptomatic control subjects who had been assessed by LD experts. The Human Investigations Committee at Massachusetts General Hospital (MGH) approved the study. The samples included acute and convalescent serum samples from 63 patients with culture-confirmed erythema migrans (EM). In addition, one pretreatment specimen was tested from each of 28 patients with active nervous system, heart, or joint involvement of the infection. All patients categorized as having LD met the CDC surveillance criteria for the diagnosis [18]. Samples were also included from 54 symptomatic patients referred for potential LD who did not meet the criteria and received a diagnosis of another illness. All phase 1 samples were derived from a set collected prospectively during 1999–2001 and stored frozen at −80°C. These samples were originally tested by other methods, and those findings are reported elsewhere [9, 11].

During phase 2, the 2-EIA algorithm was evaluated using serum samples that had been submitted to the MGH Clinical Microbiology Laboratories for LD antibody testing during a 1-year period (from 1 May 2007 through 30 April 2008). All available serum samples that had positive or equivocal results by a first-step polyvalent WCS EIA were included in the study (N = 467). On the basis of a review of medical records from the 467 patients, 78 were classified as having active LD according to the CDC case definition criteria [18]. In addition, samples were obtained from a total of 1246 healthy control subjects: 66 were collected during routine well office visits (eg, blood pressure checks) in locations in Connecticut and Rhode Island where infection is highly endemic; 1080 were collected from healthy blood donors in the Boston area, which draws from regions of endemicity, and 100 were collected from healthy blood donors in New Zealand, a region of nonendemicity. The serum samples from Boston blood donors were collected for the present study. The 66 samples from patients in Rhode Island and Connecticut were derived from a set collected prospectively during 1999–2001, and the samples from blood donors in New Zealand were collected in 2007; these were originally tested by other methods, and the findings are reported elsewhere [9, 11].

Serologic Testing
All testing was performed according to the manufacturers’ instructions. The serum samples, except those collected from Boston blood donors, were tested using the VIDAS Lyme IgG/IgM assay (bioMérieux SA). The Boston blood donor serum samples were tested using the Wampole B. burgdorferi IgG/M ELISA II assay (Alere). In addition, all samples were tested using the C6 B. burgdorferi ELISA (Immunetics). Western
immunoblotting was performed using *Borrelia* B31 IgM and IgG Virablot test strips (Viramed Biotech AG). The blots were read with the aid of densitometry; a band was defined as positive if its intensity was $\geq 90\%$ of the cutoff control band’s intensity. Western blots were interpreted using standard CDC criteria [1]. Western blotting had been performed in a previous study on the phase 1 serum samples and the serum samples from control subjects in Rhode Island, Connecticut, and New Zealand; likewise, WCS EIA had been performed on the New Zealand control serum samples [11], and the results are reported again here.

**Statistical Analysis**

Differences between proportions were considered to be statistically significant if the 2-tailed $P$ value was $\leq .05$, as determined using Fisher’s exact test.

**RESULTS**

**Phase 1: Evaluation of Well-Characterized Patients With Lyme Disease and Control Subjects**

The sensitivity of the 2-EIA algorithm was first determined using a serum set from 91 well-characterized patients with various manifestations of LD. Among 63 patients with EM (stage 1 LD), the sensitivity of the 2-EIA algorithm was 37$\%$ during the acute phase of the illness, a median of 4 days after disease onset, and 89$\%$ during convalescence, 3–4 weeks later, at the conclusion of antibiotic therapy (Table 1). In comparison, the percentages were 27$\%$ and 57$\%$, respectively, for standard 2-tiered testing. The difference in sensitivity between the 2 algorithms was statistically significant for patients in the convalescent phase of EM ($P < .001$), whereas it was not statistically significant for patients with acute EM ($P = .34$). Among 10 patients with stage 2 LD, the sensitivity of the 2-EIA algorithm was 100$\%$, compared with 40$\%$ for standard 2-tiered testing ($P = .01$). Although all 10 patients had positive results by WCS EIA, 6 with a positive IgM immunoblot result but a negative IgG immunoblot result could not be classified as positive according to CDC criteria, because the patients had been ill for $>1$ month (5–8 weeks) at the time of serum sample collection. Among the 18 patients with stage 3 LD, the sensitivity of both algorithms was 100$\%$.

The specificity of the 2-EIA algorithm was evaluated using a set of control serum samples from 54 symptomatic patients who were referred to an LD clinic for possible *B. burgdorferi* infection but who were determined to have another illness. None was positive by either the 2-EIA algorithm or the standard 2-tiered algorithm (Table 3). In contrast, 1 of 54 control specimens was positive by the C6-peptide EIA, when considered as a stand-alone test.

**Phase 2: Evaluation of Routine Serum Samples From Patients With Lyme Disease and Control Subjects**

During the 1-year study period, 4520 serum samples were submitted to the MGH Clinical Microbiology Laboratory for LD testing. Of these, 467 (10$\%$) gave positive or equivocal results in the first-step WCS EIA. After review of these patients’ medical records, it was determined that 78 (17$\%$) of 467 specimens had been collected from patients with active LD, as determined using CDC clinical criteria. Among 51 patients with acute EM, the sensitivity of the 2-EIA algorithm was 73$\%$, compared with 61$\%$ for standard 2-tiered testing ($P = .29$; Table 2). Among 16 patients with stage 2 LD, the 2-EIA algorithm was 100$\%$ sensitive, whereas standard 2-tiered testing was 94$\%$ sensitive ($P = 1.0$). All stage 2 LD samples were positive by WCS EIA and IgM or IgG Western blot, but 1 of the 16 patients had been ill for $>1$ month and had only a positive

**Table 1. Serologic Responses in Well-Characterized Lyme Disease Study Patients**

<table>
<thead>
<tr>
<th>Patients with Lyme disease (N = 91)</th>
<th>Number positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WCS EIA$^a$</td>
</tr>
<tr>
<td>Stage 1: Erythema migrans (n = 63)</td>
<td></td>
</tr>
<tr>
<td>Active phase</td>
<td>33 (52)</td>
</tr>
<tr>
<td>Convalescent phase</td>
<td>59 (94)</td>
</tr>
<tr>
<td>Stage 2: Acute neuritis or carditis (n = 10)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Stage 3: Arthritis or late neuritis (n = 18)</td>
<td>18 (100)</td>
</tr>
</tbody>
</table>

**NOTE.** WCS, whole-cell sonicate; EIA, enzyme immunoassay; WB, Western blot.

$^a$ For the EIA results, the numbers represent serum samples with either positive or equivocal results.

$^b$ In these columns, positive immunoglobulin M (IgM) Western blot results were reported regardless of the duration of illness prior to specimen collection.

$^c$ All phase 1 sera were analyzed by Western blot regardless of whether the first-step WCS EIA was positive or negative.

$^d$ In this column, IgM criteria were applied only when the duration of illness prior to specimen collection was $\leq 1$ month. The numbers represent serum samples that were positive or equivocal by WCS EIA and positive by either IgM or IgG WB analysis.

$^e$ In this column, the numbers represent serum samples that were positive or equivocal by WCS EIA and positive or equivocal by C6-peptide EIA.
Healthy control subjects (N \textsuperscript{a} regardless of whether the first-step WCS EIA was positive or negative. In contrast, sera from healthy control subjects in Boston were only tested by Western blot if the first-step WCS EIA was positive or equivocal.

The specificity of the 2-EIA algorithm was evaluated using serum samples from 1246 healthy subjects. Among the 66 well subjects living in regions where the infection is highly endemic (Connecticut and Rhode Island), the specificity of both the 2-EIA algorithm and the standard 2-tiered approach was 98%, compared with 94% for the C6 EIA alone (P = .37) (Table 3). The specificity was 99.4% for both 2-step algorithms among 1080 blood donors living in the Boston area, a region where LD is endemic, compared with 98.5% for the C6 EIA alone (P = .05). Finally, the specificity was 100% for both 2-step algorithms and for the C6 EIA alone among 100 healthy subjects from New Zealand, a region where LD is not endemic.

IgM blot result. Therefore, according to CDC guidelines, this specimen was not classified as positive by standard 2-tiered testing. Finally, among 11 patients with stage 3 LD, both algorithms were 100% sensitive.

The results from study phases 1 and 2 were similar; therefore, we combined the data for analysis. During early infection (stages 1 and 2 LD), the sensitivity of the 2-EIA algorithm was similar to that of the C6 EIA alone (61% vs 64%; P = .71) (Table 4). Both strategies were more sensitive than standard 2-tiered testing, which provided 48% sensitivity (P = .03 and P = .008, respectively). In late disease (stage 3 LD), all 3 approaches were 100% sensitive. When sensitivity was calculated using all 169 patients with active LD, irrespective of disease stage, the sensitivity of the 2-EIA algorithm was 68%, compared with 57% for standard 2-tiered testing (P = .04). The overall sensitivity of the C6 EIA was 70%, higher than either 2-step algorithm, although the difference from the 2-EIA algorithm was not statistically significant (P = .72). When all 1300 control subjects from both study phases were considered together, the

Table 2. Serologic Responses in Lyme Disease Patients Whose Sera Were Submitted to the Clinical Microbiology Laboratory at Massachusetts General Hospital

<table>
<thead>
<tr>
<th>Patients with Lyme disease (N = 78)</th>
<th>WCS EIA \textsuperscript{a}</th>
<th>C6 EIA \textsuperscript{a}</th>
<th>IgM WB \textsuperscript{b}</th>
<th>IgG WB</th>
<th>IgM and/or IgG WB \textsuperscript{b}</th>
<th>Standard 2-tiered algorithm \textsuperscript{c}</th>
<th>2-EIA algorithm \textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1: Erythema migrans (n = 51)</td>
<td>51 (100) \textsuperscript{a}</td>
<td>37 (73)</td>
<td>31 (61)</td>
<td>5 (10)</td>
<td>32 (63)</td>
<td>31 (61)</td>
<td>37 (73)</td>
</tr>
<tr>
<td>Stage 2: Acute neuritis or carditis (n = 16)</td>
<td>16 (100) \textsuperscript{a}</td>
<td>16 (100)</td>
<td>15 (94)</td>
<td>3 (19)</td>
<td>16 (100)</td>
<td>15 (94)</td>
<td>16 (100)</td>
</tr>
<tr>
<td>Stage 3: Arthritis (n = 11)</td>
<td>11 (100) \textsuperscript{a}</td>
<td>11 (100)</td>
<td>5 (45)</td>
<td>11 (100)</td>
<td>11 (100)</td>
<td>11 (100)</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} For the EIA results, the numbers represent serum samples with either positive or equivocal results.

\textsuperscript{b} In these columns, positive immunoglobulin M (IgM) Western blot results were reported regardless of the duration of illness prior to specimen collection.

\textsuperscript{c} In this column, IgM criteria were applied only when the duration of illness prior to specimen collection was \leq 1 month.

\textsuperscript{d} In this column, the numbers represent serum samples that were positive or equivocal by WCS EIA and positive or equivocal by C6-peptide EIA.

\textsuperscript{e} Patients with LD were identified by reviewing the medical record of patients with a positive first-step WCS EIA; therefore, all sera from LD patients were, by definition, positive by WCS EIA.

Table 3. Serologic Responses in Symptomatic or Healthy Control Subjects

<table>
<thead>
<tr>
<th>Number positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WCS EIA \textsuperscript{a}</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Symptomatic control subjects (N = 54) \textsuperscript{d}</td>
</tr>
<tr>
<td>Healthy control subjects (N = 1246)</td>
</tr>
<tr>
<td>Connecticut or Rhode Island sites (N = 66)</td>
</tr>
<tr>
<td>Boston blood donors (N = 1080)</td>
</tr>
<tr>
<td>New Zealand blood donors (N = 100)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} For the EIA results, the numbers represent serum samples with either positive or equivocal results.

\textsuperscript{b} All sera from symptomatic control subjects, and healthy control subjects from Connecticut, Rhode Island or New Zealand were analyzed by Western blot regardless of whether the first-step WCS EIA was positive or negative. In contrast, sera from healthy control subjects in Boston were only tested by Western blot if the first-step WCS EIA was positive or equivocal.

\textsuperscript{c} In this column, IgM criteria were applied only when the duration of illness prior to specimen collection was \leq 1 month.

\textsuperscript{d} Includes 25 patients with chronic fatigue syndrome or fibromyalgia, 14 with rheumatic diseases (such as rheumatoid arthritis or psoriatic arthritis), 9 with neurologic illnesses (including multiple sclerosis), 5 with other infections (including 2 with parvovirus B19 infection, 1 with hepatitis C infection, and 2 with presumed viral infection), and 1 with T-cell lymphoma.
specificity of the 2-EIA algorithm was 99.5%, equal to that of standard 2-tiered testing, but superior to the C6 EIA alone (specificity, 98.4%; \( P = .01 \)).

Positive Predictive Value of the Various Approaches

We calculated the positive predictive value (PPV) of the standard 2-tiered algorithm, the 2-EIA algorithm, and the C6 EIA as a stand-alone test with use of the overall sensitivity and specificity values reported in Table 4. For disease prevalence, we used 1.7%, which was the approximate prevalence of LD among those tested for the infection at our Boston hospital (4520 samples submitted for testing in 1 year; 78 patients with LD identified by medical record review). The PPV of the standard 2-tiered algorithm was 66%, compared with 70% for the 2-EIA algorithm and 43% for the C6 EIA alone (Table 5). Thus, the small reduction in specificity of the C6 EIA, compared with 2-tiered testing, resulted in a marked reduction in the predictive value of a positive test result.

DISCUSSION

We evaluated a 2-tiered approach to serologic testing for LD that used 2 EIAs, of which the first was a polyvalent WCS EIA and the second was a VlsE C6 EIA. Compared with standard 2-tiered testing, the C6 EIA alone provided greater sensitivity in early infection but less specificity. In contrast, the 2-EIA approach provided sensitivity that was very close to that of the C6 EIA alone, but maintained the specificity of standard 2-tiered testing, without the technical complexity or subjective interpretation of Western blotting.

We evaluated the 2-EIA approach with use of 2 separate serum sample sets, each with different strengths. In phase 1, the 2-EIA algorithm was evaluated using serum samples obtained in a prospective research study. The patients in this study were well characterized, including culture confirmation for the patients with EM. Therefore, there is a high degree of certainty that they were correctly classified. In phase 2, the 2-EIA system was evaluated using routine serum samples submitted to a clinical laboratory to simulate the real world situation. In both phases of the study, the 2-EIA algorithm performed better in early LD (stages 1 and 2) than did standard 2-tiered testing, whereas the algorithms were equally sensitive in late infection (stage 3). Consistent with earlier reports [6, 8, 9], the improvement in sensitivity in stage 1 resulted from use of a more sensitive C6 EIA as the second-tier assay rather than Western blotting.

In stage 2 LD, the improvement in sensitivity was attributable to an important difference in interpretive criteria between the 2 algorithms. According to the CDC criteria, a positive second-tier IgM Western blot result can be used to support the diagnosis only in persons with a duration of illness of \( \leq 1 \) month \([1]\). This

Table 4. Overall Performance of the Proposed 2-EIA Algorithm

<table>
<thead>
<tr>
<th>Patients with active Lyme disease</th>
<th>Standard 2-tiered algorithm</th>
<th>2-EIA algorithm</th>
<th>C6 EIA alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Sens (%)</td>
<td>Spec (%)</td>
<td>No.</td>
</tr>
<tr>
<td>Early Disease (N = 140)</td>
<td>67</td>
<td>48</td>
<td>–</td>
</tr>
<tr>
<td>Stage 1: Erythema migrans</td>
<td>48</td>
<td>42</td>
<td>–</td>
</tr>
<tr>
<td>Stage 2: Acute neuritis or carditis (N = 26)</td>
<td>19</td>
<td>73</td>
<td>–</td>
</tr>
<tr>
<td>Late Disease (N = 29)</td>
<td>29</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>Stage 3: Arthritis or late neuritis (N = 29)</td>
<td>96</td>
<td>57</td>
<td>–</td>
</tr>
<tr>
<td>All Patients (N = 169)</td>
<td>7</td>
<td>–</td>
<td>99.5</td>
</tr>
</tbody>
</table>

NOTE. EIA, enzyme immunoassay; No., number; Sens, sensitivity; Spec, specificity.
* The \( P \) values pertain to the comparison with the standard 2-tiered algorithm.

Table 5. Positive Predictive Value of the 3 Algorithms

<table>
<thead>
<tr>
<th></th>
<th>C6 EIA alone</th>
<th>Standard 2-tiered algorithm</th>
<th>2-EIA algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPV (%) if LD prevalence is 0.5%</td>
<td>18</td>
<td>36</td>
<td>41</td>
</tr>
<tr>
<td>PPV (%) if LD prevalence is 1.0%</td>
<td>31</td>
<td>55</td>
<td>58</td>
</tr>
<tr>
<td>PPV (%) if LD prevalence is 1.5%</td>
<td>40</td>
<td>64</td>
<td>67</td>
</tr>
<tr>
<td>PPV (%) if LD prevalence is 1.7%*</td>
<td>43</td>
<td>66</td>
<td>70</td>
</tr>
<tr>
<td>PPV (%) if LD prevalence is 2.0%</td>
<td>47</td>
<td>70</td>
<td>74</td>
</tr>
</tbody>
</table>

NOTE. EIA, enzyme immunoassay; LD, Lyme disease; PPV, positive predictive value.
* The numbers in this row, which are highlighted in bold font, indicate the PPV values calculated using the prevalence of LD among those tested at our hospital (1.7%), as determined in phase 2 of this study. The other PPV values were calculated using hypothetical prevalence rates, for comparison.
caveat was included to reduce the number of false-positive results, because patients were expected to have positive IgG antibody responses after 1 month of illness. Although patients with stage 2 LD often have both IgM and IgG reactivity with early antigens, such as the 23-, 39-, and 41-kD spirochetal proteins, it may take up to 2 months before they have IgG responses against 5 antigens, as required for a positive IgG blot result [11]. In the current study, 7 of 26 patients with stage 2 LD had only positive IgM responses 5–8 weeks after disease onset; therefore, they were classified as seronegative by standard 2-tiered testing, reducing the clinical sensitivity of the standard algorithm. In contrast, the proposed 2-EIA algorithm is meant to be applied to any patient, regardless of duration of illness. This modification improves sensitivity in stage 2 LD, compared with standard 2-tiered testing, and it obviates the need to pinpoint the exact duration of illness, which can be difficult.

Certainly, there is a downside to the 2-EIA algorithm, compared with Western blotting. With blots, one learns the spirochetal antigens against which the patient’s antibody response is directed, and the response expands over time. This gives information about the duration of illness, and in cases in which the diagnosis is difficult, this information may be helpful. Thus, there is still a need for Western blotting in the armamentarium of serologic tests for LD. However, in routine cases, this level of detail is often superfluous and prone to misinterpretation.

Does the 2-EIA approach give enough additional information to justify the added labor and expense, compared with use of the C6 EIA alone? Although the 2-EIA algorithm was slightly less sensitive in stage 1 LD than the C6 EIA alone (53% vs 56%; \( P = .69 \)), serologic testing is not recommended for stage 1 disease [19], and the 2 approaches were equally sensitive in stages 2 and 3. This small difference in sensitivity was offset by a statistically significant difference in specificity, which favored the 2-EIA algorithm over the C6 EIA alone (99.5% vs 98.4%; \( P = .01 \)). This difference in specificity translates into large differences in PPV (70% vs 43% in our study), because the prevalence of LD in tested populations is usually low, even in areas of endemicity. Furthermore, reducing specificity by 1%, as was shown here, changes the false-positive rate from 0.5% to 1.6%.

In the United States, where at least 3.4 million LD tests are done annually [20], this difference would lead to an additional 37,000 false-positive results per year. Thus, the number of false-positive results would exceed the reported incidence of LD in the United States (>35,000 cases annually) [21]. This finding, in our opinion, is the reason to retain a 2-test approach.

One could also consider variations of the 2-EIA strategy. One could reverse the order of the 2 tests, starting with the C6 EIA and, in those with a positive or equivocal result, follow with a WCS EIA. This would increase the number of C6 tests while reducing the number of WCS EIA tests. However, because the C6 test is more expensive and more prone to quality control failures, we prefer the order of performing the WCS EIA first. Alternately, one could do both EIAs at the same time, rather than in step-wise fashion. Such an approach could be adapted to a simpler format, such as a lateral flow immunochromatographic test strip, potentially allowing for sensitive and specific diagnosis of LD at the point of care.

Our study had several limitations. First, testing was done at different times and using frozen samples. However, all specimens were stored at \(-80^\circ\text{C}\), and freeze-thaw cycles were minimized. Second, samples were not tested from control subjects with other tickborne infections, and further study is warranted. Third, control serum samples from the MGH blood bank were tested using a different WCS EIA than the other study specimens. However, both WCS EIAs use the same antigen target (B. burgdorferi strain B31), and both are FDA-cleared and performed similarly in FDA trials.

In summary, a 2-tiered antibody test for LD that consists of a WCS EIA followed by a VlsE C6 EIA performs as well as, and in some respects better than, the standard 2-tiered approach. The 2-EIA algorithm provided sensitivity comparable to that of the C6-peptide EIA alone but maintained equivalent specificity, compared with standard 2-tiered testing. Thus, for routine testing, the standard 2-tiered algorithm could be replaced by 2 EIAs.

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Potential conflicts of interest. A. C. S. has received a research grant from Viramed Biotech AG in the past, to support an earlier study. J. A. B. has received a research grant from Diasorin, to support a separate study. M. J. F. has been a member of the scientific advisory board at bioMé rieux. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed in the Acknowledgments section.

References


