MAJOR ARTICLE

A Comparison of Lyme Disease Serologic Test Results From 4 Laboratories in Patients With Persistent Symptoms After Antibiotic Treatment

Brian A. Fallon,[1] Martina Pavlicova,[2] Samantha W. Coffino,[3] and Carl Brenner[4]

Departments of [1]Psychiatry, [2]Biostatistics, Mailman School of Public Health, [3]Neurology, Columbia University, and [4]Lamont-Doherty Earth Observatory of Columbia University, Palisades, New York

(See the Editorial Commentary by Dattwyler and Arnaboldi on pages 1711–3.)

Background. As the incidence of Lyme disease (LD) has increased, a number of "Lyme specialty laboratories" have emerged, claiming singular expertise in LD testing. We investigated the degree of interlaboratory variability of several LD serologic tests—whole cell sonicate (WCS) enzyme-linked immunosorbent assay (ELISA), immunoglobulin M (IgM) and immunoglobulin G (IgG) Western blots (WBs), and an ELISA based on the conserved sixth region of variable major protein–like sequence expressed (C6)—that were performed at 1 university laboratory, 1 commercial laboratory, and 2 laboratories that specialize in LD testing.

Methods. Serum samples from 37 patients with posttreatment Lyme syndrome, as well as 40 medically healthy controls without prior LD, were tested independently at the 4 laboratories.

Results. In general, there was little difference among the laboratories in the percentage of positive test results on the ELISAs and IgG WBs, although the number of discordant results was often high. When in-house criteria for positivity were used at the 2 specialty laboratories, specificity at 1 laboratory declined considerably on both the IgM and IgG WBs. The Centers for Disease Control and Prevention (CDC) 2-tiered criteria improved overall concordance. At the 2 laboratories that performed the C6 ELISA, the percentage of positive tests was comparable to that of the WCS ELISA while providing higher specificity. The IgM WB performed poorly in our patient population of individuals with later-stage illness, a result consistent with previous studies.

Conclusions. Although there was surprisingly little difference among the laboratories in percentage of positive results on most assays using CDC criteria, interlaboratory variability was considerable and remains a problem in LD testing.

Keywords. Lyme; *Borrelia burgdorferi*; serology; diagnosis.

Lyme disease (LD) is a multisystem, tick-transmitted disease caused by the spirochete genogroup *Borrelia burgdorferi* sensu lato. Two species, *Borrelia afzelii* and *Borrelia garinii*, are responsible for most cases of European Lyme disease [1], whereas the overwhelming majority of LD infections in the United States are caused by *B. burgdorferi* sensu stricto [2].

In patients with erythema migrans (EM) and recent exposure to an endemic area, the diagnosis of LD can be made clinically. In patients with later disseminated disease, however, serologic testing takes on increased importance, as many late manifestations of LD (eg, meningitis, cranial neuropathy, arthritis, and encephalopathy) are nonspecific [3]. The Centers for Disease Control and Prevention (CDC) has developed a 2-tiered diagnostic algorithm for LD in the United States, consisting of a sensitive whole cell sonicate screening assay—for example, enzyme-linked immunosorbent assay (ELISA) or indirect immunofluorescence assay—followed by immunoglobulin M (IgM) or immunoglobulin G (IgG) Western blot (WB) testing of positive or equivocal screened samples [4]. Current CDC criteria for

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Correspondence: Brian A. Fallon, MD, NYS Psychiatric Institute, Rm 3724, Unit/Box 69, 1051 Riverside Drive, New York, NY 10032 (baf1@columbia.edu).

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a positive WB require the presence of 2 (of 3) specified bands on the IgM WB or 5 (of 10) specified bands on the IgG WB.

Although ELISA testing is more objective than reading and interpreting WBs, several studies have shown considerable interlaboratory variability with both methods, due in part to lack of test standardization and the subjectivity associated with Western blot interpretation [5–8]. The emergence of "Lyme specialty laboratories" has introduced an additional variable to this picture. In addition to occasional differences in testing methodology, some of these laboratories provide 2 sets of criteria for a positive test, 1 based on the CDC recommendations and the other devised by the laboratory itself. Some patients and clinicians believe that an ELISA or WB obtained through a Lyme specialty laboratory may be more sensitive than comparable tests performed at a national commercial laboratory or academic center.

In this study we compared ELISA, IgG WB, and IgM WB results from 4 laboratories—1 university-based laboratory, 1 commercial laboratory, and 2 Lyme specialty laboratories— to assess (1) whether there was significant interlaboratory variability and (2) if qualitative performance differences among the laboratories were apparent. We also examined results from the 2 specialty laboratories that performed ELISAs based on the highly conserved sixth region (C6) of the variable major protein–like sequence expressed (VlsE) lipoprotein of *B. burgdorferi* [9–11].

PATIENTS AND METHODS

Subjects

Samples from patients and controls were derived from specimens obtained during the conduct of 2 research protocols approved by the institutional review board of the New York State Psychiatric Institute, for which all patients signed informed consent. Both studies enrolled individuals with posttreatment Lyme syndrome (PTLS). The first, conducted from 1999 to 2005, recruited patients and controls for an antibiotic retreatment study that required patients to have historical evidence meeting CDC surveillance criteria for LD as well as a positive IgG Western blot from a single university-based reference laboratory (UBRL) at the time of study screening; the methods and results of this study have been previously published [12]. The second study, conducted from 2005 to 2007, recruited patients and controls both for this laboratory investigation and for a study of single photon emission computed tomography brain scans among patients with a history of LD and non–medically ill controls. Although patients in this study were required to have met historical clinical and laboratory criteria for LD, they were not required to have a positive IgG WB at screening. Control subjects met the following criteria: (1) no history of prior diagnosis or treatment for LD; (2) no history of Lyme-like symptoms or illness (eg, chronic fatigue syndrome, fibromyalgia, arthritic disorder, peripheral neuropathy); (3) no history of another major neurologic or medical disorder; and (4) lack of residence in or recent exposure to a highly Lyme-endemic area.

Of the 37 subjects with LD and 20 healthy controls enrolled in the first study, serum samples remained from 26 patients and 7 controls for inclusion in this study. From the second study, samples were available from 11 Lyme patients and 33 controls, for a total of 37 patients and 40 controls in this investigation. Twenty-four of the 37 Lyme patients (64.9%) were female, and the mean age of Lyme patients was 46.5 years (SD, 10.5 years). Twenty-four of the 40 control subjects (60%) were female, and controls had a mean age of 43.9 years (SD, 11.7 years).

Samples for Laboratory Tests

Serum samples from patients and controls were sent for Lyme ELISA and IgM and IgG Western blot assays to 4 different laboratories, masked as to LD or control group status. Of these 4 laboratories, 1 was the UBRL, 1 was a nonspecialty commercial laboratory, and 2 were Lyme specialty laboratories (hereafter referred to as Laboratories A and B). For exploratory purposes, Lyme C6 peptide ELISA was also performed at the Lyme specialty laboratories. Archived samples were kept in a −80°C freezer and unthawed until testing.

Statistics

A McNemar χ^2 test was used to compare paired patient test results of the UBRL to each of the other laboratories. When a specialty laboratory reported results using both the CDC criteria and internal laboratory criteria, each set of criteria was compared separately to the UBRL. Tables 1 and 2 report the number and percentage of positive tests for both the PTLS cohort and controls, and the number of discordant results between the UBRL and each of the other laboratories. Results were considered significant if the corresponding *P* value was smaller than level of significance $\alpha = .001$. A level of significance of 0.1% was selected to account for the multiple comparisons evaluated.

RESULTS

ELISA and IgG WB

In the cohort of 37 PTLS patients, all of the laboratories had a similar percentage of positive results on the ELISA, although the number of discordant pairs between the UBRL and the other laboratories was considerable, ranging from 14 at Laboratory A to 12 at the commercial laboratory to 8 at Laboratory B (Table 1). Using CDC criteria for the interpretation of the IgG WB, the UBRL had the highest percentage of positive results at 56.8%, whereas the percentage of positives at the other laboratories ranged from 43.2% to 48.6%. The number of discordant pairs between the UBRL and each of the other laboratories on the IgG WB was similar.

Table 1. Number and Percentage of Positive Serologic Test Results and Discordant Pairs for 37 Posttreatment Lyme Syndrome Patients (University Reference Laboratory Versus Commercial and Lyme Specialty Laboratories)

	University Reference Laboratory	Commercial Laboratory			Specialty Laboratory A			Specialty Laboratory B		
Test	No. Positive[a] (%)	No. Positive[a] (%)	*P* Value	Discordant Pairs	No. Positive[b] (%)	*P* Value	Disc Pairs	No. Positive[c] (%)	*P* Value	Disc Pairs
?/+ ELISA	23 (62.2)	25 (67.6)	.773	12	25 (67.6)	.789	14	25 (67.6)	.724	8
C6 ELISA	. . .	. . .	. . .	. . .	25 (67.6)	. . .	. . .	23 (62.2)	. . .	. . .
WB IgM (CDC)	8 (21.6)	6 (16.2)	.724	8	1 (2.7)	.016	7	16 (43.2)	.027	10
WB IgM (laboratory)	. . .	. . .	. . .	. . .	1 (2.7)	.016	7[d]	23 (62.2)	<.001	15[d]
WB IgG (CDC)	21 (56.8)	16 (43.2)	.074	5	16 (43.2)	.074	5	18 (48.6)	.250	3
WB IgG (laboratory)	. . .	. . .	. . .	. . .	14 (37.8)	.016	7[d]	26 (70.3)	.131	7[d]
2-tier: ?/+ ELISA & WB IgG	18 (48.6)	15 (40.5)	.250	3	14 (37.8)	.220	6	16 (43.2)	.688	6
2-tier: C6 ELISA & WB IgG	. . .	. . .	. . .	. . .	15 (40.5)	. . .	. . .	17 (45.9)	. . .	. . .
2-tier: ?/+ ELISA & C6 ELISA	. . .	. . .	. . .	. . .	22 (59.5)	. . .	. . .	18 (48.6)	. . .	. . .

Abbreviations: ?/+, indeterminate/positive; CDC, Centers for Disease Control and Prevention; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IgM, immunoglobulin M; WB, Western blot.

[a] The university-based reference laboratory and commercial laboratory used CDC criteria for all WB tests. Criteria for a positive IgM WB were ≥2 of the following bands: Osp C, 39, 41. Criteria for a positive IgG WB were ≥5 of the following bands: 18, Osp C, 28, 30, 39, 41, 45, 58, 66, 93.

[b] In-house laboratory criteria for a positive IgM WB at Specialty Laboratory A were ≥2 of the following bands: 23, 39, 41, 83/93. Criteria for a positive IgG WB were ≥3 of the following bands: 20, 23, 31, 34, 35, 39, 83/93.

[c] In-house laboratory criteria for a positive IgM WB at Specialty Laboratory B were ≥2 of the following bands: 23–25, 31, 34, 39, 41, 83/93. Criteria for a positive IgG WB were ≥2 of the following bands: 23–25, 31, 34, 39, 41, 83/93.

[d] Results using in-house criteria at Specialty Laboratories A and B were compared with results using CDC criteria at the university-based reference laboratory.

Using the CDC 2-tiered algorithm of a positive or equivocal ELISA followed by an IgG WB, the UBRL had a 48.6% positivity rate, whereas positivity at the other laboratories ranged from 37.8% to 43.2%. Compared with the ELISA, the 2-tiered algorithm reduced the number of discordant pairs between the UBRL and each of the other laboratories. The 2 specialty laboratories also reported internal, non-CDC interpretive criteria for IgG WBs (see Table 1 footnotes). Using these in-house criteria, the percentage of positive IgG WB results dropped at Laboratory A from 43.2% to 37.8% but rose at Laboratory B from 48.6% to 70.3%.

Specificity on the ELISA was highest at Laboratory A (97.5%), and ranged from 87.5% to 92.5% at the other laboratories (Table 2). Using CDC interpretive criteria for the IgG WB, the commercial laboratory and Laboratory A had no false positives, whereas the UBRL had 1 and Laboratory B had 3. When in-house interpretive criteria were used, the number of false positives at Laboratory B rose to 11 (27.5%). Specificity using the CDC 2-tiered algorithm was 100% at all laboratories except Laboratory B, which had 1 false positive.

C6 ELISA

The 2 specialty laboratories also performed C6 ELISA assays; positivity was 67.6% at Laboratory A and 62.2% at Laboratory B. Specificity was 100% at both laboratories. Using a 2-tiered approach combining an initial positive C6 ELISA with an IgG WB, Laboratories A and B had positive rates of 40.5% and 45.9%, respectively. Based on the results of recent studies [13], it has been postulated that a 2-tiered strategy consisting of an initial whole cell immunoassay followed by a VlsE C6 peptide enzyme immunoassay may provide greater sensitivity than the conventional 2-tiered strategy without sacrificing specificity [14]. As a result, although our study's focus was not on determining the optimum algorithm for LD testing, we examined this strategy for the 2 laboratories that performed C6 studies and obtained positive rates of 59.5% for Laboratory A and 48.7% for Laboratory B. Specificity remained 100% at both laboratories. These figures represented an increase in positivity over the conventional 2-tiered strategy at Laboratory B, but fell short of the C6 test on its own at both laboratories.

IgM WB

Although IgM WB testing is not recommended by the CDC for patients with an illness duration of >1 month, given its frequent use in the community we also report IgM results. Using CDC interpretive criteria, there was 21.6% positivity at the UBRL, whereas positivity ranged from 2.7% to 43.2% at the other laboratories. Specificity using CDC interpretive criteria was 100%

Table 2. Number and Percentage of False-Positive Serologic Test Results and Discordant Pairs for 40 Medically Healthy Controls (University Reference Laboratory Versus Commercial and Lyme Specialty Laboratories)

Test	University Reference Laboratory	Commercial Laboratory			Specialty Laboratory A			Specialty Laboratory B		
	No. Positive[a] (%)	No. Positive[a] (%)	*P* Value	Disc Pairs	No. Positive[a] (%)	*P* Value	Disc Pairs	No. Positive[a] (%)	*P* Value	Disc Pairs
?/+ ELISA	5 (12.5)	3 (7.5)	.683	6	1 (2.5)	.125	4	3 (7.5)	.683	6
C6 ELISA	. . .	. . .	. . .	. . .	0	. . .	. . .	0	. . .	. . .
WB IgM (CDC)	5 (12.5)	0	.074	5	1 (2.5)	.125	4	8 (20.0)	.505	9
WB IgM (laboratory)	. . .	. . .	. . .	. . .	1 (2.5)	.125[b]	4	15 (37.5)	.024	16[b]
WB IgG (CDC)	1 (2.5)	0	1.00	1	0	1.00	1	3 (7.5)	.480	2
WB IgG (laboratory)	. . .	. . .	. . .	. . .	0	1.00[b]	1	11 (27.5)	.004	10[b]
2-tier: ?/+ ELISA & WB IgG	0	0	. . .	0	0	. . .	0	1 (2.5)	1.000	1
2-tier: C6 ELISA & WB IgG	. . .	. . .	. . .	. . .	0	. . .	. . .	0	. . .	. . .
2-tier: ?/+ ELISA & C6 ELISA	. . .	. . .	. . .	. . .	0	. . .	. . .	0	. . .	. . .
+ WB IgM or IgG (CDC)	5 (12.5)	0	.074	5	1 (2.5)	.133	4	10 (25.0)	.182	9
+WB IgM or IgG (laboratory)					1 (2.5)	.133	4	23 (57.5)	<.001	22

Abbreviations: ?/+, indeterminate/positive; CDC, Centers for Disease Control and Prevention; Disc pairs, discordant pairs; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IgM, immunoglobulin M; WB, Western blot.

[a] Criteria for a positive test are given in Table 1.

[b] Results using in-house criteria at Specialty Laboratories A and B were compared with results using CDC criteria at the university-based reference laboratory.

at the commercial laboratory and ranged from 80% to 97.5% at the other laboratories. When in-house interpretive criteria for IgM WBs were used, there was no change in results for Laboratory A but the percentage of positive tests at Laboratory B rose from 43.2% to 62.2% for the 37 PTLS patients, whereas specificity dropped from 80% to 62.5%.

DISCUSSION

The emergence of specialty laboratories for LD testing has resulted in sometimes sharp disagreement among physicians about their quality. We attempted to determine if there was significant interlaboratory variability and/or qualitative differences among a university-based laboratory, a commercial laboratory, and 2 Lyme specialty laboratories. Because the university-based laboratory was used to determine eligibility for patients enrolled in the first of the 2 earlier studies, we designated it as the reference laboratory for comparison in this paper.

Among the tests recommended for later stage disease (ELISA and IgG WB), there was no evidence that any 1 laboratory outperformed the others in detecting serum antibodies, as the percentage of positive results from PTLS patients was comparable across laboratories. (Positivity was nonsignificantly higher at the UBRL, but no performance-related significance can be inferred given that this was the reference laboratory used to determine eligibility for enrollment into the first study). There were, however, a considerable number of discordant pairs between the UBRL and other laboratories on all of the tests, particularly among the PTLS patients; thus, patient serum samples may test positive at one laboratory but not another. Among PTLS patients, this discordance was prominent for the ELISA (ranging from 8 to 14 samples) and the IgM WB (ranging from 7 to 10 samples), and somewhat less prominent for the IgG WB (ranging from 3 to 5 samples). The generally low ELISA and IgG WB positivity figures for all laboratories were likely a function of the study population; because all patients in our study had been previously treated with antibiotics and because the duration of illness was variable and spanned many years for some, active infection cannot be presumed and lack of test positivity does not represent a failure of the test. Thus, while this study design evaluates the concordance among laboratories on rates of positivity, it does not test assay "sensitivity."

For the ELISA, there were small, nonsignificant differences in the number of false-positive results among healthy controls across laboratories, ranging from 2.5% to 12.5%. Specificity for the IgG WB was somewhat better than the ELISA, with false-positive rates across laboratories of 0%–7.5%. Discordant pairs were also fewer among the controls, ranging from 4 to 6 for the ELISA and 1–2 for the IgG WB.

Using the CDC 2-tiered algorithm for ELISA and IgG WB, positivity was comparable (37.8%–48.7%) across the laboratories, although marginally higher at the UBRL—again, likely reflecting its status as reference laboratory for the first study. Even so, there was no statistically significant difference in positivity

between the UBRL and any of the other laboratories. Furthermore, despite the relatively high number of discordant results between the UBRL and the other laboratories on the ELISA, the use of the 2-tiered algorithm brought the final results into closer alignment.

Differences in antigenic composition were likely the main driver of interlaboratory variability on the ELISA. Although all of the laboratories in our study used whole cell sonicates of *B. burgdorferi* for antibody detection, not all used the same kit. There is significant variability among whole cell sonicate kits, and sometimes even within lots of the same kit [15]. Most kits detect some combination of IgG and IgM (and in some cases IgA) antibodies, but detection of specific immunoglobulin classes, especially IgM, can be highly variable. The interlaboratory variability (and relatively poor specificity) seen in ELISA results has long plagued LD testing [5–8, 15]; the 2-tiered system was designed in part to address this shortcoming. More recently, novel tests based on recombinant antigens and/or synthetic peptides have been developed. These newer assays are now in wider use; indeed, the commercial laboratory in our study has since switched to an ELISA that utilizes a dual combination of recombinant VlsE-1 and synthetic pepC10 IgG and IgM antigens [16].

Different kits were also employed by the study laboratories for WB testing. The UBRL and Laboratory B used kits developed in-house, whereas the commercial laboratory and Laboratory A used the Marblot kit developed by MarDx Diagnostics. Laboratory B's in-house WB kit used strips from a mixture of 2 strains of *B. burgdorferi*. Despite the differences in kits, IgG WB positivity was similar across all laboratories. However, only the 2 laboratories using commercial kits attained 100% specificity on the IgG WB, indicating that the in-house kits may suffer from a relative deficiency in specificity. Specificity at the 2 laboratories using in-house kits improved using the CDC 2-tiered criteria, however, to 100% at the UBRL and 97.5% at Laboratory B.

Because some Lyme specialty laboratories report both the CDC and their own in-house criteria for WB interpretation, clinicians may be uncertain as to which set of criteria are preferable. The in-house criteria for Laboratories A and B, given in Table 1, were generally less stringent than the CDC guidelines, requiring fewer bands to be considered positive and expanding or modifying the list of diagnostically significant bands, although at Laboratory A the in-house criteria also involved removing some bands considered significant by the CDC. IgG WB positivity at Laboratory A declined marginally using their own criteria, from 43.2% to 37.8%, while specificity remained at 100% and percentage discordance remained unchanged. At Laboratory B, positivity increased using in-house criteria, from 48.6% to 70.3%, but specificity declined to a poor 72.5%.

C6 ELISA positivity was very similar at Laboratories A and B. Both laboratories had 100% specificity, and concordance between these 2 laboratories was good (only 2 discordant pairs). Overall, the C6 ELISA alone had a higher positivity rate with equal or better specificity than any of the 2-tiered testing algorithms we examined.

National and international academic committees do not recommend the IgM WB for diagnosis beyond the first month of infection, primarily because many treated patients will express an IgM response for an extended period even after symptom resolution and because false-positive results may occur due to other medical conditions such as infectious mononucleosis or syphilis [17–21]. The significance of a persistent IgM response has been debated, but in our patient population of individuals with longstanding symptoms after treatment, this test performed poorly. Using CDC criteria, IgM positivity was quite low—21.6% at the UBRL and 2.7%–43.2% across the other laboratories. The commercial laboratory had no false-positive results, but specificity at the other laboratories was variable, and particularly poor (80%) at Laboratory B. The use of in-house criteria at Laboratory A did not change IgM WB positivity or specificity, but at Laboratory B it further decreased specificity to 62.5%.

Patients and physicians sometimes interpret a positive result on either the IgM or IgG WB among PTLS patients as a reliable marker of past or current infection. We examined how the laboratories performed using this "combined" approach (Table 2). Laboratory A retained good specificity (97.5%) using either CDC or in-house criteria, but Laboratory B showed a decline in specificity to 75% using CDC criteria and a further decline to 42.5% using in-house criteria, implying that more than half of people without LD are at risk of inappropriate antibiotic treatment when this laboratory's in-house criteria are used as the primary basis for diagnosis. These results underscore the high variability in laboratory specificity, particularly when in-house criteria are used, and do not support the use of a "combined" approach.

Our study has several possible limitations. First, because the sample size was small, it was likely underpowered to validly detect possible differences between the UBRL and other laboratories that might have become apparent with a larger patient population. However, we were able to definitively address our primary aim of assessing whether there was notable interlaboratory variability among the laboratories on most of the tests. Second, the use of the university-based laboratory as a reference laboratory for the first study made it impossible to draw useful inferences from its IgG WB performance in comparison to the other laboratories, but this too had no effect on the fundamental issue of assessing interlaboratory variability. Third, our patient population consisted of patients with significant longstanding symptoms after treatment, and thus is probably not representative

either of acute LD cases or of most cases that clinicians see in their practices. However, our results are consistent with previous studies showing that interlaboratory variability in LD serologic testing remains a common phenomenon. Fourth, although it is conceivable that our medically healthy volunteers included individuals previously unknowingly infected with *B. burgdorferi*, we think it highly unlikely that this was more than a rare occurrence.

In light of the relatively high level of discordance among laboratories, some clinicians may consider sending patient serum samples to a second laboratory if a case of LD is highly suspected but not confirmed by initial testing; however, this practice should be restricted to those laboratories demonstrated to have good specificity on these tests. The justification for such a strategy should rest upon an awareness of the decline in the positive predictive value of a test when specificity is poor, when clinical history suggests LD is unlikely, and when an individual has not been exposed to a Lyme-endemic area [3, 22, 23].

Notes

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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.

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