MAJOR ARTICLE

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

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(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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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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Comparison of Lyme disease serologic assays and "Lyme specialty laboratories"

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Dear Editor:

The paper by Fallon, Pavlicova, Coffino, and Brenner in this journal, addresses an important issue, do "Lyme specialty laboratories" offer an advantage in the sero-diagnosis of Lyme disease (LD)? To put this question in context, it helps to have an appreciation of the history of the laboratory diagnosis of LD. The discovery of *Borrelia burgdorferi* (Bb) in 1982 (1) allowed for the development of sero-assays to detect antibodies to this pathogen (2-7). By the late 1980's, it was clear that the lack of specificity and standards needed to be confronted (2). The CDC in collaboration with a group of university based researchers set about to address these problems. The result of this effort was the development of a paradigm, commonly known as the "CDC criteria". This paradigm was established in 1995 as the standard for performing and interpreting Lyme disease serologies in the United States (2). It is a two-tier system (typically an ELISA followed by a Western Blot) that has generally worked well and successfully dealt with the major problem of specificity. However, it has a number of shortcomings: it is more costly than a single tier system, it is insensitive in early infection and the second tier western blot is confusing to many practitioners (3-11). The latter two issues have contributed to the poor repute of LD sero-assays in general and a common but false impression that there is a delay in the human immune response to *B. burgdorferi*. In truth, the immune response to *B. burgdorferi* is similar to those of other bacterial infections; specific IgM is detectable within 7 days of infection and IgG a few days later (12).

Diminished sensitivity in early disease is the direct result of the limited number of antigens recognized and the antigen targets themselves (12-16). FlaB, (41 kd flagellin), OspC and IR6, the peptide from the 6th conserved region of VlsE (the basis of the C6 peptide assay), are early antigens that are each illustrative of problems with serologic target antigens currently in use. FlaB cross reacts with many other bacterial flagellins and a high percentage of healthy non-Bb infected individuals have antibody reactivity with this antigen (2,3,17). OspC, though less cross-reactive, has 24 serotypes (18,19). VlsE (IR6), the least cross-reactive, is only expressed after infection is established (less than 1% of Bb in the feeding tick express this antigen), IR6 only contains a single human epitope, does not bind IgM well and has more variability than originally thought (20-22). Though the impression that LD serologies are insensitive seems to a generalization from problems in early infection. The reality is that sensitivity is less of a concern in late disease because of the broader number of Bb antigens recognized. Nonetheless, simpler, more sensitive and specific assays are needed.

Despite a great deal of effort to improve LD sero-diagnostics over the past 2 decades, there have been only incremental improvements. Many laboratories and assay manufacturers are still using cultured Bb as the source of antigen targets in their assays. The fundamental problem with the use of whole protein Bb antigens is that they express epitopes that are both unique to Bb as well as

epitopes similar to those expressed by other bacteria (2,17). Whether based on whole cultured Bb, recombinant proteins or single peptide antigens, fundamental issues of sensitivity and specificity and the balance between the two remain for all current LD sero-assays.

It is in this environment that there have arisen clinical laboratories that claim special expertise and greater test sensitivity in the laboratory diagnosis of Lyme disease. These laboratories often employ their own independently developed 'in-house' assays and unique interpretation of western blot results. Independent studies evaluating the effectiveness of specialized testing at these laboratories are lacking. The study by Fallon, Pavlicova, Coffino, and Brenner in this journal, addresses an important issue, do Lyme specialty laboratories offer an advantage in the sero-diagnosis of LD. The authors compared the results of Lyme disease testing by ELISA using sonicates of cultured Bb as the antigen source, IgM and IgG western blots. The tests were carried out at a university-based laboratory, a large commercial laboratory, and two "Lyme disease specialty laboratories." All western blots were evaluated using standard criteria and the two "Lyme disease specialty laboratories" also interpreted the western blots using their own criteria. The two "Lyme specialty laboratories" also evaluated the samples using the C6 peptide ELISA. The patient population used were patients with post-treatment Lyme disease syndrome. In late LD, IgG immune responses predominate. Measurement of IgM responses is not recommended after the first 4 weeks of infection such as in these patients. The control population was healthy adults.

In the study population, when the standard 2-tier paradigm was performed, the sensitivity of the assays was similar and none of the laboratories stood out. Thus, there was no significant advantage in using any of the laboratories. In contrast, when interpreting the western blot results by their own criteria, the results changed for both "Lyme specialty laboratories". Specialty laboratory A saw a reduction in sensitivity, while for Specialty laboratory B sensitivity increased almost two-fold. This increase occurred when they applied their own interpretation to the WB. Thus, one could claim that their methods provided greater sensitivity. However, this increase in sensitivity came at what can only be considered an unacceptable price, a steep decrease in specificity, 15 of 40 (37.5%) of the normal healthy controls met their IgM criteria, 11 of the 40 (27.5%) met their IgG criteria and 23 of the 40 healthy controls (57.5%) met one or the other.

This study not only demonstrates that "Lyme specialty laboratories" offered no advantage but it provides a lesson in laboratory medicine. Many seem to have forgotten that like other serologic assays, Lyme serologies are not by themselves diagnostic. This raises a major issue, how should serologies be used and what is the ability of Lyme serologies to correctly predict if a person does or does not have Lyme disease. For any test, predictive value has to be considered. In the case of Lyme disease, positive predictive value refers to the ability for a positive serology to correctly identify someone with Lyme disease and negative predictive value refers to the ability for a negative serology to correctly identify someone without Lyme disease. Both of these values are directly related to the pre-test likelihood of having the disease. A negative serology in a patient with only nonspecific complaints without

the objective clinical abnormalities associated with Lyme disease and a low pre-test likelihood of disease is very highly predictive that the patient does not have Lyme disease. But what is the meaning of a positive serology in patients with the same nonspecific complaints? There are physicians who routinely order LD serologic assays in patients primarily based on nonspecific complaints, including fatigue, stiff neck, arthralgia, myalgia, palpitations, abdominal pain, sleep disturbance, poor concentration, irritability, depression, back pain, headache, dizziness, or other nonspecific symptoms. Is there utility in doing this? All of these nonspecific symptoms are commonly reported in otherwise healthy members of the general population (20) With 20-25% of the population having nonspecific complaints, the positive predictive value of a positive serology using CDC criteria, IgG only for complaints of over 4 weeks, is extremely poor. It is certainly not diagnostic. For serologies with specificities like those of reported for "Lyme specialty laboratory" B, a "positive serology" in this patient population has such a low positive predictive value that it has virtually no value. Simply stated, basing a diagnosis of Lyme disease or any other tick-borne infectious disease on the presence of one or more of these common vague symptoms is unjustified.

It is obvious that alternatives to current assays and the CDC criteria are needed. However, changes require rigorous scientific validation and in the absence of valid proof, "new criteria" are simply unacceptable. Nonspecific assays and serologies in patients with a low pre-test likelihood of LD are a combination that does more harm than good. High positive predictive value requires good assay specificity and a high pre-test likelihood of LD.

As in Fallon et al published in this issue, C6 ELISA has clearly demonstrated that a peptide containing a specific epitope can improve both improved sensitivity and specificity when compared to whole protein based assays. However, the C6 assay has limitations that have precluded its adoption as a stand-alone assay. Improved serologic assays are needed and it is likely that a multi-peptide assay based on peptides containing specific epitopes from multiple key Bb antigens could solve many of the issues of current LD sero-diagnosis. Until such an assay is developed, the CDC criteria and good laboratory practice should continue to be the standard. With regard to assays with poor specificity, to paraphrase George Santayana, those who cannot remember the past are condemned to repeat it. We should all remember that poor specificity was a major issue that led to the adoption of the two- tier paradigm in the first place.

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Comparison of Lyme Disease Serologic Assays and Lyme Specialty Laboratories

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(See the Major Article by Fallon et al on pages 1705–10.)

Keywords. Lyme disease; *Borrelia burgdorferi*; serodiagnosis; Western blot; predictive value.

The article by Fallon et al in this issue of *Clinical Infectious Diseases* addresses an important issue: Do "Lyme specialty laboratories" offer an advantage in the serodiagnosis of Lyme disease (LD)? To put this question into context, it helps to have an appreciation of the history of the laboratory diagnosis of LD. The discovery of *Borrelia burgdorferi* in 1982 [1] allowed for the development of seroassays to detect antibodies to this pathogen [2–7]. By the late 1980s, it was clear that the lack of specificity and standards needed to be confronted [2]. The Centers for Disease Control and Prevention (CDC), in collaboration with a group of university-based researchers, set about to address these problems. The result of this effort was the development of a paradigm, commonly known as the "CDC criteria." This paradigm was established in 1995 as the standard for performing and interpreting LD serologies in the United States [2]. It is a 2-tier system (typically an enzyme-linked immunosorbent assay [ELISA] followed by a Western blot) that has generally worked well and successfully dealt with the major problem of specificity. However, it has a number of shortcomings: It is more costly than a single-tier system, it is insensitive in early infection, and the second-tier Western blot is confusing to many practitioners [3–11]. The latter 2 issues have contributed to the poor repute of LD seroassays in general and a common but false impression that there is a delay in the human immune response to *B. burgdorferi*. In truth, the immune response to *B. burgdorferi* is similar to those of other bacterial infections; specific immunoglobulin M (IgM) is detectable within 7 days of infection and immunoglobulin G (IgG) a few days later [12].

Diminished sensitivity in early disease is the direct result of the limited number of antigens recognized and the antigen targets themselves [12–16]. FlaB, (41-kd flagellin), OspC, and IR6, the peptide from the sixth conserved region of VMP like sequence (VlsE) (the basis of the C6 peptide assay), are early antigens that are each illustrative of problems with the serologic target antigens currently in use. FlaB cross-reacts with many other bacterial flagellins, and a high percentage of healthy non–*B. burgdorferi*–infected individuals have antibody reactivity with this antigen [2, 3, 17]. OspC, although less cross-reactive, has 24 serotypes [18, 19]. VlsE (IR6), the least cross-reactive, is only expressed after infection is established (<1% of *B. burgdorferi* in the feeding tick express this antigen); IR6 only contains a single human epitope, does not bind IgM well, and has more variability than originally thought [20–22], although the impression that LD serologies are insensitive seems to be a generalization from problems in early infection. The reality is that sensitivity is less of a concern in late disease because of the broader number of *B. burgdorferi* antigens recognized. Nonetheless, simpler, more sensitive, and more specific assays are needed.

Despite a great deal of effort to improve LD serodiagnostics over the past 2 decades, there have been only incremental improvements. Many laboratories and assay manufacturers are still using cultured *B. burgdorferi* as the source of antigen targets in their assays. The fundamental problem with the use of whole-protein *B. burgdorferi* antigens is that they express epitopes that are both unique to *B. burgdorferi* as well as epitopes similar to those expressed by other bacteria [2, 17]. Whether based on whole cultured *B. burgdorferi*, recombinant proteins, or single-peptide antigens, fundamental issues of sensitivity and specificity and the balance between the 2 remain for all current LD seroassays.

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It is in this environment that there have arisen clinical laboratories that claim special expertise and greater test sensitivity in the laboratory diagnosis of LD. These laboratories often employ their own independently developed "in-house" assays and unique interpretation of Western blot results. Independent studies evaluating the effectiveness of specialized testing at these laboratories are lacking. The study by Fallon et al in this issue of *CID* addresses an important issue—namely, do Lyme specialty laboratories offer an advantage in the serodiagnosis of LD? The authors compared the results of LD testing by ELISA using sonicates of cultured *B. burgdorferi* as the antigen source, as well as IgM and IgG Western blots. The tests were carried out at a university-based laboratory, a large commercial laboratory, and 2 LD specialty laboratories. All Western blots were evaluated using standard criteria, and the 2 LD specialty laboratories also interpreted the Western blots using their own criteria. The 2 LD specialty laboratories also evaluated the samples using the C6 peptide ELISA. The patient population used were patients with post-treatment LD syndrome. In late LD, IgG immune responses predominate. Measurement of IgM responses is not recommended after the first 4 weeks of infection, such as in these patients. The control population was healthy adults.

In the study population, when the standard 2-tier paradigm was performed, the sensitivity of the assays was similar and none of the laboratories stood out. Thus, there was no significant advantage in using any of the laboratories. In contrast, when interpreting the Western blot results by their own criteria, the results changed for both LD specialty laboratories. Specialty laboratory A saw a reduction in sensitivity, whereas for specialty laboratory B, sensitivity increased almost 2-fold. This increase occurred when they applied their own interpretation to the Western blot. Thus, one could claim that their methods provided greater sensitivity. However, this increase in sensitivity came at what can only be considered an unacceptable price, a steep decrease in specificity: 15 of 40 (37.5%) of the normal healthy controls met their IgM criteria, 11 of the 40 (27.5%) met their IgG criteria, and 23 of the 40 healthy controls (57.5%) met one or the other.

This study not only demonstrates that LD specialty laboratories offered no advantage, but it also provides a lesson in laboratory medicine. Many seem to have forgotten that, like other serologic assays, LD serologies are not by themselves diagnostic. This raises a major issue—namely, how should serologies be used, and what is the ability of Lyme serologies to correctly predict if a person does or does not have LD? For any test, predictive value has to be considered. In the case of LD, positive predictive value refers to the ability for a positive serology to correctly identify someone with LD, and negative predictive value refers to the ability for a negative serology to correctly identify someone without LD. Both of these values are directly related to the pretest likelihood of having the disease. A negative serology in a patient with only nonspecific complaints, without the objective clinical abnormalities associated with LD and a low pretest likelihood of disease, is very highly predictive that the patient does not have LD. But what is the meaning of a positive serology in patients with the same nonspecific complaints? There are physicians who routinely order LD serologic assays in patients primarily based on nonspecific complaints, including fatigue, stiff neck, arthralgia, myalgia, palpitations, abdominal pain, sleep disturbance, poor concentration, irritability, depression, back pain, headache, dizziness, or other nonspecific symptoms. Is there utility in doing this? All of these nonspecific symptoms are commonly reported in otherwise healthy members of the general population [23, 24]. With 20%–25% of the population having nonspecific complaints, the positive predictive value of a positive serology using CDC criteria, IgG only for complaints of >4 weeks, is extremely poor. It is certainly not diagnostic. For serologies with specificities like those of reported for Lyme specialty laboratory B, a "positive" serology in this patient population has such a low positive predictive value that it has virtually no value. Simply stated, basing a diagnosis of LD or any other tick-borne infectious disease on the presence of ≥1 of these common vague symptoms is unjustified.

It is obvious that alternatives to current assays and the CDC criteria are needed. However, changes require rigorous scientific validation, and, in the absence of valid proof, "new" criteria are simply unacceptable. Nonspecific assays and serologies in patients with a low pretest likelihood of LD are a combination that does more harm than good. High positive predictive value requires good assay specificity and a high pretest likelihood of LD.

As in Fallon et al's article, C6 ELISA has clearly demonstrated that a peptide containing a specific epitope can improve both improved sensitivity and specificity when compared to whole protein-based assays. However, the C6 assay has limitations that have precluded its adoption as a stand-alone assay. Improved serologic assays are needed, and it is likely that a multipeptide assay based on peptides containing specific epitopes from multiple key *B. burgdorferi* antigens could solve many of the issues of current LD serodiagnosis. Until such an assay is developed, the CDC criteria and good laboratory practices should continue to be the standard. With regard to assays with poor specificity, to paraphrase George Santayana, "those who cannot remember the past are condemned to repeat it." We should all remember that poor specificity was a major issue that led to the adoption of the 2-tier paradigm in the first place.

Note

Potential conflict of interest. R. D. is an employee of and has received royalties from the Biopeptides Corporation, and has received grants

from the National Institutes of Health. P. A. is an employee of the Biopeptides Corporation.

Both 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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Lyme disease: How reliable are serologic results?

Dan Gregson MD, G. Evans MD, David Patrick MD, William Bowie MD

See also, www.cmaj.ca/lookup/doi/10.1503/cmaj.141413

Lyme disease is a tick-transmitted bacterial infection that is well established in North America. It is uncommon in most areas of Canada, but its incidence and geographic range are increasing. The accurate diagnosis of Lyme disease is critical to ensure that those patients who truly have the condition are given appropriate antibiotics. Furthermore, an accurate diagnosis ensures patients with nonspecific symptoms are not mistakenly told that they have Lyme disease. In their recent practice article,[1] Andany and colleagues discuss a clinical scenario in which a Canadian man pursued testing for Lyme disease through a commercial laboratory in the United States. The test showed a positive result that was at odds with serologic testing conducted through a public health laboratory.[1]

This patient scenario illustrates for readers that American specialty laboratories should not be considered to provide a more sensitive assay for the diagnosis of Lyme disease than their public health counterparts. Recent research has documented a high rate of false-positive results with extremely poor positive predictive value in some specialty laboratories.[2] Mistakes in diagnosis can deprive patients of treatment specific to the true cause of their symptoms, and can result in prolonged therapy for a condition they do not have.

The methods for diagnosing *Borrelia burgdorferi* infection, the organism that causes Lyme disease, have been continuously improving since the microbe was first discovered in 1982.[3] The method involves the use of European *Borrelia* species and flagellar antigens in the screening serology to improve the sensitivity or negative predictive value, and confirmatory Western blotting assays to increase the specificity and positive predictive value of the test.[4,5] Some Lyme disease advocacy groups espouse that Centers for Disease Control and Prevention (CDC) criteria used for the serologic diagnosis of Lyme disease are inadequate, and they recommend alternative interpretive standards.[6] However, a recent study by Fallon and colleagues[2] formally evaluated how current testing algorithms work in two patient groups and several types of laboratories in the US. The findings support previous conclusions of the CDC[7] and highlight two important lessons for physicians and consumers.

In a well-defined cohort of patients with post-treatment symptoms of Lyme disease, tests done in a university or commercial laboratory using well-defined CDC criteria for the serologic diagnosis of Lyme disease were as sensitive as testing done in laboratories specializing in Lyme testing. This remained true even when the specialty laboratories used in-house criteria to "increase" the sensitivity of their Western blot testing. Accordingly, such laboratories cannot be considered to be better at picking up infections missed by standard CDC criteria.

Furthermore, 40 patients without Lyme disease were included in the study as a negative control group. The inclusion of immunoglobulin M in the interpretation of control group Western blot samples led to false-positive results from three of the four laboratories studied (a rate of 2.5%–25%). One specialty laboratory using in-house criteria (immunoglobulins G or M) had false-positive results in 57% of the samples from the negative control group.

Fallon and colleagues' study further dispels the myth that US specialty laboratories provide a more sensitive assay for the diagnosis of Lyme disease, and documents a high rate of false-positive results with poor positive predictive values in some specialty laboratories. As a conse-

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KEY POINTS

- The serologic diagnosis of Lyme disease in Canada is best done using standard laboratory protocols as implemented by the National Microbiology Laboratory of Canada using criteria recommended by the Centers for Disease Control and Prevention.
- Recent evidence suggests that standard assays and testing algorithms used in Canada are as sensitive as those used in American specialty laboratories for detecting infection with *Borrelia burgdoferi*.
- Specialty laboratory tests have a high rate of false-positive results owing to their use of non–evidence based interpretation criteria, particularly when results rely solely on Western blot analysis.
- Most Canadians who are told that they have Lyme disease based solely on results from specialty laboratory typically have other causes for their symptoms.

quence, patients and physicians should be cautious in choosing a referral laboratory in the US when seeking "second opinion" serology after receiving a negative test result in Canada. Laboratories that use the standard CDC two-tier testing algorithms should be preferred over those that report results based on unproven, unvalidated, in-house criteria. Any positive result from a test that relies solely on Western blotting is most likely a false-positive.

Patients with chronic subjective symptoms without a diagnosis can be vulnerable and desperate for an answer as to the cause of their illness. Giving them a false diagnosis based on flawed testing is misleading. Inappropriate therapy based on such results leads to economic, psychological and physical adverse outcomes.[8–10] Rather, these patients deserve a complete and accurate evaluation to detect illnesses for which appropriate interventions can be applied and, whatever their diagnosis, supports to improve the quality of life for themselves and their families.

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DECISIONS

A 35-year-old man with a positive Lyme test result from a private laboratory

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See also www.cmaj.ca/lookup/doi/10.1503/cmaj.150874

A 35-year-old man with a 12-month history of fatigue is concerned about Lyme disease. He has not lived in or travelled to a Lyme endemic area. His physical examination and blood work, including complete blood count, electrolytes, creatinine, liver enzymes and thyroid function tests, are normal. Lyme serology conducted by a provincial public health laboratory has a negative result. The patient undertakes Lyme testing through a commercial laboratory in the United States, which shows a positive result. He asks his physician if he should be given antibiotics for Lyme disease.

Are the patient's symptoms consistent with Lyme disease?

Lyme disease is the most common vector-borne infection in North America[1,2] and incidence in Canada is increasing, with more than 500 cases reported in 2013.[3] However, this patient is very unlikely to have Lyme disease because he lacks both a compatible epidemiologic exposure and clinical findings of Lyme infection.

The cause of Lyme disease, *Borrelia burgdorferi*, is transmitted to humans through the bite of infected blacklegged ticks (*Ixodes scapularis* or *Ixodes pacificus*).[1] Most infections occur during spring and summer, and transmission is uncommon if tick attachment is less than 36 hours.[1] Infected ticks have now established endemic populations in several Canadian provinces, including British Columbia, Manitoba, Ontario, Quebec and Maritime provinces.[3]

The signs and symptoms of Lyme disease are categorized into three stages (Box 1).[1] Untreated Lyme disease may progress to later stages of infection.[1] The term "chronic Lyme disease" is sometimes used to describe a number of later stage syndromes, but lacks a consistent definition. It is occasionally applied to cases in which patients have late neurologic Lyme disease — neuroborrelosis — but such patients have active infection and fall within stage 3. Similarly, some patients with untreated Lyme arthritis will have symptoms for months or years, but are also classified in stage 3.[4] A subset of patients with confirmed and appropriately treated Lyme disease will have persistent symptoms beyond six months in the absence of objective clinical findings, which is termed post–Lyme disease syndrome.[1,4]

Why does this patient have discrepant test results for Lyme disease?

Lyme disease can be diagnosed either clinically or by appropriate serologic testing. A clinical diagnosis can be made in patients with erythema migrans and plausible exposure.[1,5] Because this patient does not report a rash at the onset of his illness, he would require a positive serologic test result for diagnosis. However, only patients with compatible objective findings and a reasonable epidemiologic exposure should undergo testing. Testing is discouraged for patients with nonspecific symptoms owing to the poor predictive value and possibility of false-positives.[1,5]

The standard test for Lyme disease consists of a two-tier strategy performed in accredited laboratories[1] in accordance with recommendations from the Infectious Diseases Society of America (IDSA) and the Centers for Disease Control and Prevention (CDC),[1,2] and is the approach employed by Canadian public health laboratories.[5] Enzyme immunoassay is used for the initial screening. If the result is negative, no further testing is done. If the result is positive or equivocal, a Western blot is used for confirmation of the results.[1,2] The results are interpreted in accordance with CDC guidelines. Antibodies typically develop within four to six weeks, at which point sensitivity of the two-step protocol is about 87% and specificity is about 99%.[6] False-negative results may occur

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The clinical scenario is fictional.

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Box 1: Clinical signs and symptoms of Lyme disease[1,4]

Stage of disease	Syndrome	Description
1	Erythema migrans	• Large, red lesion with central clearing ("target lesion") at the site of the initial tick bite • May be accompanied by fever, headache and joint pain
2	Neurologic Lyme disease (neuroborrelosis)	• Cranial nerve palsy, with or without meningitis • Lyme meningitis • Lyme radiculopathy
	Cardiovascular Lyme disease	• Heart block • Myocarditis • Pericarditis
3	Arthritis	• One or multiple joints at a time
	Late neurological Lyme disease	• Encephalopathy • Encephalitis • Peripheral neuropathy
	Acrodermatitis chronica atrophicans	• Skin discolouration and swelling that can occur up to 8 years after initial infection
	Post–Lyme disease syndrome	• Subjective symptoms after treatment without objective clinical findings

in early stage disease, when the sensitivity of the two-step protocol is about 40%.[6]

Clinicians should be aware that several commercial laboratories offer Lyme testing, but that the diagnostic techniques used are unvalidated and have poor test characteristics (e.g., urine antigen tests, CD57 antigen testing, polymerase chain reaction [PCR] testing, immunoblot with in-house interpretation not adherent to CDC standards).[2] Molecular detection methods for Lyme disease, such as PCR testing, are problematic for several reasons, including the complexity of *Borrelia burgdorferi*'s antigenic composition, the sparse bacterial loads in clinical samples, the differences in assays and genetic targets and their limited clinical validation.[7] In addition, PCR techniques are subject to contamination, and false-positives have been reported.[8] Thus, antibody detection has become the mainstay of diagnosing Lyme disease.

The utility of Western blot testing requires standardized methods and interpretation of the results.[9] The current guidelines for interpreting Western blot results recommended by the CDC are based on the systematic evaluation of these diagnostic tools. During acute infection (within 4 weeks), both immunoglobulin M and G immunoblots are required; infection beyond four weeks should be evaluated with immunoglobulin G immunoblot only.[9] These recommendations take into account the variability in interpretation and the reduction in specificity when immunoglobulin M immunoblots are done in cases where illness has lasted for more than 1 month.[5] The performance of the Western blot when used and interpreted outside of these criteria is unclear.[4]

Should antimicrobial therapy be offered to this patient?

Treatment recommendations for Lyme disease are outlined in the IDSA guideline[1] and vary with the stage of infection. This patient does not have objective clinical evidence of early Lyme infection (i.e., erythema migrans), which is the only stage of infection that should be treated empirically. In all other cases of suspected Lyme disease, infection should be documented with serology based on standardized testing protocols before any treatment is started. Antimicrobial therapy should not be offered to patients with chronic subjective symptoms but negative serology results from a public health laboratory, such as this patient.[1] Symptoms may persist for months or years after appropriate treatment in patients with post–Lyme disease syndrome. However, evidence from randomized controlled trials has shown that prolonged antibiotic therapy is associated with no or minimal benefit and increased adverse events in these patients.[1,4]

The case revisited

This patient has not resided in a Lyme endemic area and lacks objective clinical or validated laboratory findings of Lyme infection. He should be counselled that the methods used by the private laboratory are nonstandardized and may lack the appropriate reliability and validity to establish a diagnosis, and that the testing method used in public health laboratories is the current accepted

standard. Antimicrobial therapy is not recommended and could potentially lead to adverse events, including *Clostridium difficile* infection and vascular catheter-associated complications (were the patient to receive antimicrobial agents intravenously). If there had been a history of potential Lyme exposure in the last four to six weeks, testing at a public health laboratory could be repeated to look for convalescent serology; otherwise, the patient should undergo evaluation for alternative causes for his symptoms.

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Decisions is a series that focuses on practical evidence-based approaches to common presentations in primary care. The articles address key decisions that a clinician may encounter during initial assessment. The information presented can usually be covered in a typical primary care appointment. Articles should be no longer than 650 words, may include one box, figure or table and should begin with a very brief description (75 words or less) of the clinical situation. The decisions addressed should be presented in the form of questions. A box providing helpful resources for the patient or physician is encouraged.

MAJOR ARTICLE

Poor Positive Predictive Value of Lyme Disease Serologic Testing in an Area of Low Disease Incidence

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Background. Lyme disease is diagnosed by 2-tiered serologic testing in patients with a compatible clinical illness, but the significance of positive test results in low-prevalence regions has not been investigated.

Methods. We reviewed the medical records of patients who tested positive for Lyme disease with standardized 2-tiered serologic testing between 2005 and 2010 at a single hospital system in a region with little endemic Lyme disease. Based on clinical findings, we calculated the positive predictive value of Lyme disease serology. Next, we reviewed the outcome of serologic testing in patients with select clinical syndromes compatible with disseminated Lyme disease (arthritis, cranial neuropathy, or meningitis).

Results. During the 6-year study period 4723 patients were tested for Lyme disease, but only 76 (1.6%) had positive results by established laboratory criteria. Among 70 seropositive patients whose medical records were available for review, 12 (17%; 95% confidence interval, 9%–28%) were found to have Lyme disease (6 with documented travel to endemic regions). During the same time period, 297 patients with a clinical illness compatible with disseminated Lyme disease underwent 2-tiered serologic testing. Six of them (2%; 95% confidence interval, 0.7%–4.3%) were seropositive, 3 with documented travel and 1 who had an alternative diagnosis that explained the clinical findings.

Conclusions. In this low-prevalence cohort, fewer than 20% of positive Lyme disease tests are obtained from patients with clinically likely Lyme disease. Positive Lyme disease test results may have little diagnostic value in this setting.

Keywords. *Borrelia burgdorferi*; Lyme; serology; positive predictive value; diagnostic testing.

Lyme disease is a tick-borne zoonotic bacterial infection caused by *Borrelia burgdorferi* sensu lato. It is the most common vector-borne infectious disease in the temperate northern hemisphere, reported in tens of thousands of residents of the United States each year [1]. Lyme disease most commonly presents with a distinctive erythema migrans (EM) skin lesion, but if untreated the disease can disseminate to other organ systems, causing arthritis, meningitis, cranial and peripheral neuropathy, and cardiac conduction abnormalities. These syndromes are not unique to Lyme disease, and in the absence of the characteristic EM rash, serologic testing is necessary to differentiate Lyme disease from other conditions [2].

Lyme disease transmission is geographically heterogeneous, however, and for any given clinical presentation the likelihood of Lyme disease will be influenced by regional disease prevalence. This is primarily a function of tick populations, particularly the density of host-seeking nymphal black-legged ticks infected with *B. burgdorferi* [3, 4]. States and regions where infected ticks are uncommon have low transmission rates of Lyme disease. North Carolina, for example, has a low annual incidence of Lyme disease (<0.5 cases per 100 000 population), and entomologic data suggest there is a very low risk of human Lyme

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disease there [3, 5, 6]. In endemic regions, such as the northeastern and upper Midwestern United States, Lyme disease is responsible for an appreciable burden of meningitis, arthritis, and cranial neuropathy. By contrast *B. burgdorferi* infection will be responsible for a much smaller proportion of these syndromes in areas with little or no Lyme disease transmission. Considering that the pretest probability of a disease strongly influences interpretation of any diagnostic test result, we hypothesized that positive Lyme disease test results will be less meaningful in regions with low disease prevalence. To this end, we performed a large cross-sectional retrospective study of patients undergoing evaluation for Lyme disease presenting to clinics and hospitals located in an area with little Lyme disease transmission.

METHODS

Study Design

We performed a retrospective study of electronic medical records for adults and children evaluated at both inpatient and outpatient sites in the Duke University Health System between 1 January 2005 and 31 December 2010. The institutional review board approved the study protocol with a waiver of informed consent.

Patient Identification and Data Abstraction

We queried the electronic medical records to identify 2 cohorts of patients: those with a positive 2-tiered Lyme disease serologic test result and those tested for Lyme disease during a compatible illness (Figure 1). For the first cohort, we reviewed the electronic medical records to determine whether each patient had a clinical presentation compatible with active Lyme disease documented within 1 month of when the diagnostic test was obtained. We abstracted testing results, information about their clinical presentation, documentation of an alternative diagnosis, and documentation of tick exposure in a Lyme disease endemic state.

For the second cohort, we focused on patients with oligoarticular arthritis of large joints, meningitis, and cranial nerve palsy. Although these syndromes do not encompass the full clinical spectrum of disseminated Lyme disease, we selected the conditions that are most frequently attributable to Lyme disease in endemic areas [7–16]. We searched for patients who had been tested for Lyme disease and whose record contained *International Classification of Diseases, Ninth Revision* diagnostic codes, *Current Procedure Terminology* 4 procedure codes, or laboratory codes compatible with arthritis, meningitis, or cranial neuropathy (Supplementary Table 1). We then reviewed the medical records to confirm documentation of arthritis, meningitis, or cranial neuropathy at the time of Lyme disease testing.

To identify each cohort we performed queries of the Duke electronic medical records. Our search terms identified inpatients and outpatients of all ages tested at Duke-affiliated laboratories. We excluded patients from both cohorts without available electronic medical records to review. All patients were tested using the Meridian Premier Lyme EIA kit (catalog Nos. 696016 and 696032). Specimens reactive by this kit were then tested by Western blot using the Trinity Biotech

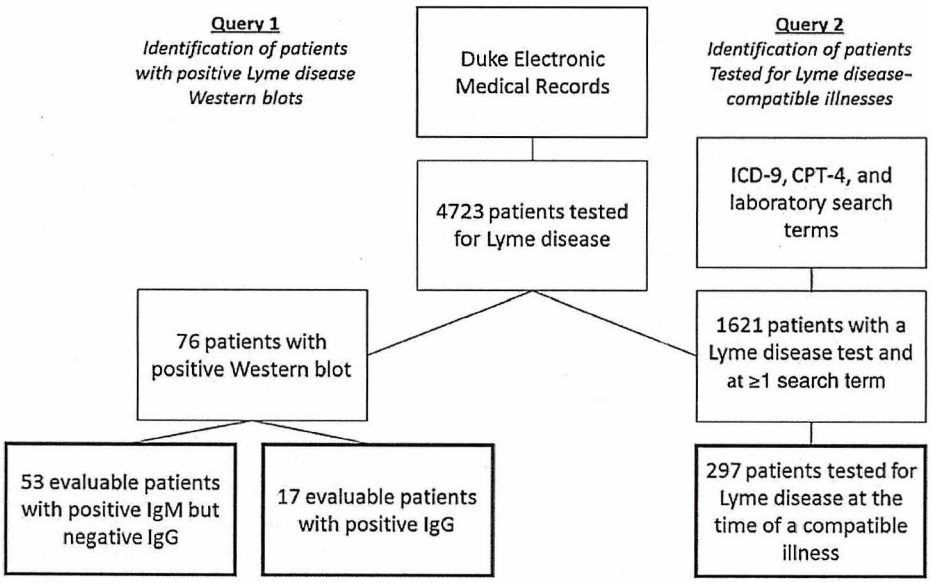

Figure 1. Workflow used to conduct electronic medical record queries. Abbreviations: CPT-4, *Current Procedure Terminology* 4; ICD-9, *International Classification of Diseases, Ninth Revision*; IgG, immunoglobulin G; IgM, immunoglobulin M.

B. burgdorferi IgG and IgM MarBlot Strip Test systems (catalog Nos. 40–2075 G and 40–2075 M).

Outcome Measure

We defined a case of Lyme disease as the coexistence of a positive 2-tiered Lyme disease serologic test and a compatible clinical illness. This is in accordance with recommended clinical and diagnostic practices, definitions accepted for Lyme disease clinical trials, as well as the Centers for Disease Control and Prevention surveillance definition of Lyme disease [17–19]. A positive 2-tiered test is conventionally defined as positive or equivocal results of an enzyme-linked immunosorbent assay (ELISA) using a *B. burgdorferi* whole-cell sonicate, followed by a positive immunoglobulin (Ig) M or IgG Western immunoblot, defined as ≥2 of 3 reactive IgM bands or ≥5 of 10 reactive IgG bands [20]. The Duke University Health System clinical laboratories perform ELISA followed by automatic Western blot analysis in the event of positive or equivocal ELISA results. Individual band results are not reported to clinicians. We classified patients who were seropositive by IgM criteria as "false-positive" if they did not have a positive IgG Western blot within 2 months of symptom onset [19, 20]. Although conventionally a 1-month cutoff is recommended, beyond which the IgM results should no longer be considered [3], we chose 2 months given the difficulty of precisely dating symptom onset in a retrospective study.

We classified seropositive patients as "true-positive" if they had chart documentation of any of the following clinical presentations: EM-like skin lesions, large-joint arthritis (including clinical or radiographic documentation of a joint effusion or inflammatory synovial fluid), meningitis (documented by elevated lymphocyte counts in the cerebrospinal fluid [CSF]), motor cranial neuropathy, radiculopathy or peripheral neuropathy, or atrioventricular block (documented by electrocardiography). Patients with an alternative diagnosis that explained their syndrome were reclassified as false-positives.

Statistical Analysis

We calculated the positive predictive value by dividing the true-positives by total positives (true-positives plus false-positives) [21]. We calculated 95% confidence intervals (CIs) around proportions using standard binomial distributions. For all statistical analysis, we used Stata 13.1 statistical software (StataCorp).

RESULTS

During the study period, clinicians ordered 5756 Lyme disease serologic tests for 4723 unique patients; 229 patients were tested ≥2 times. Among the 4723 tested patients, 76 were positive by 2-tiered testing (1.6% of patients; 95% CI, 1.2%–2.0%). Among 70 patients with accessible medical records, 53 were positive by IgM Western blot criteria alone, and 17 patients were positive by IgG Western blot criteria (with or without also meeting IgM criteria).

Among the 17 evaluable subjects who were positive by IgG Western blot criteria (Table 1), 5 were judged to be true-positives by virtue of syndromes characteristic of Lyme disease. One had a peripheral facial nerve palsy, 2 had knee effusions, 1 had a knee

Table 1. Patients With Positive Lyme Disease Test Results by 2-Tier IgG Criteria

Patient Sex	Clinical Presentation[a]	Alternative Diagnosis	Geographic Exposure
Male	Arthralgias	. . .	. . .
Male	Polyarthralgias	Celiac-associated joint pain	. . .
Male*	Facial nerve palsy		Connecticut
Male*	Knee effusion		Maryland
Male	Arthralgia, history of Lyme disease	Repetitive stress	. . .
Male*	Arthritis		. . .
Male	Chronic pain	. . .	. . .
Female	Fever, urticaria, hand swelling	Allergic drug reaction	. . .
Female	Fever, headache, fatigue, negative CSF results	. . .	. . .
Female	Visual field loss	Retinal lesions	. . .
Male	High fever while in Southeast Asia	. . .	. . .
Male	Trigeminal neuralgia	. . .	. . .
Male*	Polyarthritis, sausage digit		. . .
Male	Skin lesions	Eosinophilic lichenoid dermatitis	. . .
Male	Cranial nerve III palsy	Metastatic cancer to cavernous sinus	. . .
Male	Uveitis	. . .	. . .
Male*	Knee effusion and TMJ crepitus		. . .

Abbreviations: CSF, cerebrospinal fluid; IgG, immunoglobulin G; TMJ, temporomandibular joint.

[a] Arthralgia was defined as joint pain or stiffness without documentation of joint effusion or inflammation; arthritis, as joint pain or stiffness with such documentation. Cases judged as "true positive" are marked with an asterisk (*).

effusion as well as arthritis of the temporomandibular joint, and 1 had polyarthritis that included the knee but also (uncharacteristically for Lyme disease) "sausage" digits (however, this patient responded to antibiotic therapy, and no alternative diagnosis was made). Two of the 5 true-positive patients had documented travel to known Lyme disease–endemic regions with potential tick exposure (Connecticut and Maryland). One patient was classified as false-positive based on an alternative medical diagnosis, cranial nerve palsy caused by carcinoma metastatic to the cavernous sinuses. In addition, 1 patient with positive results had isolated uveitis, and another had isolated trigeminal neuralgia. Neither of these conditions is known to be associated with Lyme disease in the absence of other more characteristic manifestations of the infection [22–25]. The remaining 9 patients had syndromes incompatible with Lyme disease and/or an alternative diagnosis (Table 1). Three seropositive patients had histories of Lyme disease, but lacked findings consistent with active infection at the time of the test.

Among the 53 evaluable subjects who were positive only by IgM criteria, 8 had syndromes compatible with active Lyme disease (15%; 95% CI, 5%–25%). These included 5 individuals with EM-like skin lesions, 1 with a CSF pleocytosis, 1 with facial nerve palsy, and 1 with a knee effusion (Supplementary Table 2). One individual had first-degree atrioventricular block but had presented with a high fever, elevated hepatic transaminases, absolute monocytopenia, and hyponatremia and had a clinical diagnosis of human monocytic ehrlichiosis. Among the remaining subjects who met IgM criteria, 18 had been symptomatic for ≥2 months without positive IgG results, 24 had clinically incompatible illnesses lacking objective findings of Lyme disease, and 3 patients were asymptomatic. Four of the 8 patients with true-positive IgM results had documentation of exposure in highly endemic states: Rhode Island, Connecticut, New York, and New Jersey; for 3 of them, the exposure was clearly recent.

Overall, 12 of 70 patients who met 2-tiered testing criteria had an illness compatible with active Lyme disease at the time of the test. Without considering travel history, the positive predictive value of a 2-tiered serologic testing was 17% (95% CI, 9%–28%). At least 6 of 12 patients with true-positive results had most likely acquired their disease during travel to endemic regions. Excluding patients with a history of travel to a Lyme disease endemic area, would leave 6 true-positive results in 59 cases, yielding a positive predictive value of 10% (95% CI, 2%–18%) in the nonendemic region studied.

We then identified patients who had been tested for Lyme disease in the setting of a clinical illness compatible with Lyme disease. Applying the search criteria described in the methods section yielded 2569 medical encounters for 1621 unique patients. Of these, 297 patients (18%) had a Lyme disease serologic test at the time of a clinically compatible illness; 110 patients had arthritis of a large joint, 98 had cranial nerve palsy, 75 had meningitis, 11 had both meningitis and cranial neuropathy, and 1 patient each had arthritis with cranial nerve palsy, atrioventricular block alone, or atrioventricular block with cranial nerve palsy (Table 2).

Table 2. Patients With Select Lyme Disease–Compatible Presentations Identified Through Electronic Medical Record Queries

Presentation	Patients (Female/Male), No.	Positive Test Results[a]	Age Mean (Range), y
Arthritis	110 (59/51)	3	31.1 (2–91)
Meningitis	75 (44/31)	1	43.8 (4–82)
CN	98 (49/49)	1	46.8 (7–84)
Meningitis plus CN	11 (4/7)	1	36.5 (8–71)
Other[b]	3 (1/2)	0	48 (38–58)

Abbreviation: CN, cranial neuropathy.

[a] Positive tests results were defined according to standard 2-tier interpretive criteria, including a reactive enzyme-linked immunosorbent assay followed by positive immunoglobulin M or G Western immunoblot.

[b] Atrioventricular block, atrioventricular block plus cranial nerve palsy, and arthritis plus cranial nerve palsy in 1 patient each.

Of these 297 patients, 6 tested positive for Lyme disease by 2-tiered serologic testing, 3 by IgG and 3 solely by IgM criteria. These 6 patients had also been identified in our search of all seropositive patients. Three of the 6 had effusions of large joints at the time of presentation; 1 had CSF pleocytosis, 1 had peripheral facial nerve palsy, and 1 had both facial nerve palsy and CSF pleocytosis. Thus, the prevalence of Lyme disease among patients was (at most) 6 of 297 (2%, 95% CI, .7%–4.3%). Three of these 6 seropositive individuals had documented recent travel to Lyme disease–endemic areas, where the infection was probably acquired (Maryland, Connecticut and Massachusetts). The patient with facial nerve palsy and CSF pleocytosis ultimately received a diagnosis of central nervous system vasculitis associated with anti-neutrophil cytoplasmic antibodies. Thus, if we exclude these 4 patients, only 2 of 297 cases (0.7%; 95% CI, .08%–2.4%) were likely to be cases of locally acquired Lyme disease.

DISCUSSION

In a region where Lyme disease is uncommon, even patients with highly characteristic clinical presentations are rarely found to have Lyme disease, and positive test results are seldom associated with clinically probable infection. Indeed, among 70 patients with positive tests during a 6-year period, only 13 had an illness compatible with Lyme disease. Only a small minority of seropositive patients with clinical presentations compatible with disseminated Lyme disease were likely to have acquired the infection disease locally. Our findings raise the question of whether positive Lyme disease test results have diagnostic value in low-prevalence regions, such as North Carolina. With a high background noise of false-positive test results,

coupled with a low signal of true-positive cases, it may be impossible to trust a positive result.

Serologic testing for Lyme disease is most useful for patients who have an intermediate pretest probability of infection [2]. Patients in endemic areas with characteristic EM-like skin lesion skin findings do not require testing, because they are highly likely to have Lyme disease, and there is significant likelihood of a false-negative test [26]. At the other end of the spectrum, patients with a low pretest probability of Lyme disease are more likely to have a false-positive or nonexplanatory positive test result. These include individuals with no objective manifestations of Lyme disease, including those who have only nonspecific symptoms (eg, fatigue) and those who probably have an alternative diagnosis [27]. They also include patients who live in nonendemic areas and have not traveled to endemic areas, even if their symptoms are compatible with Lyme disease.

Previous studies have shown that patients with objective clinical findings consistent with disseminated Lyme disease have an intermediate likelihood of Lyme disease that will maximize the yield of diagnostic testing. For instance Lyme disease accounts for 22%–34% of facial nerve palsy cases [12, 15], 13%–28% of meningitis cases in children [7, 8, 11, 14], and 31%–67% of monoarthritis cases in children [9, 10, 13, 16]. We must emphasize, however, that these studies were all conducted in regions of the Northeast with exceptionally heavy transmission of Lyme disease. The patients in these studies had both intermediate clinical and epidemiologic risk of *B. burgdorferi* infection.

We must remember that the coexistence of a positive serologic test and a consistent clinical illness does not absolutely prove that a patient has Lyme disease. Lyme IgM Western blots, in particular, produce many false-positive results [28]. With roughly 3 million Lyme disease tests ordered annually, even a specificity of 99% would yield tens of thousands of false-positive results. A background prevalence of false-positive results can coincidentally overlap with a background prevalence of Lyme disease mimics, resulting in misdiagnosis of Lyme disease in patients with other diagnoses. Arthritis has been diagnosed in >20% of American adults, for example [29]. At the same time, seroreactivity to *B. burgdorferi* occurs out of proportion to the incidence of clinically apparent Lyme disease, and asymptomatic infection is well documented [30–32]. Awareness of epidemiologic context and the absence of an alternative diagnosis are necessary for a clinician to decide whether a positive test is explanatory or coincidental [33]. On the other hand, the negative predictive value of Lyme disease testing will be very high in a region with low prevalence, and in a region where Lyme disease is emerging, a negative results may provide patients with some reassurance.

Our study has several limitations. First, it was retrospective, and we were limited to the data recorded in the medical record. Travel histories in particular were recorded briefly and seldom described the intensity of exposure to tick habitat during travel; moreover, the *absence* of travel was almost never specifically documented. Judging the compatibility of each patient's syndrome with Lyme disease depended on a retrospective review of chart documentation, rather than a prospectively defined case definition, and thus individual cases may have been misclassified because of inadequate documentation. To improve the specificity of our study, we selected common manifestations of disseminated Lyme disease that were easily identified by diagnostic and procedural coding, and elected not to perform queries for rarer manifestations, such as carditis. Our aim was not to capture all patients with possible Lyme disease but rather to identify patients with the maximum clinical pretest probability.

We also cannot be sure that our patient cohort is generalizable to all patients in North Carolina, because we conducted our study in a health system that includes a large academic tertiary care center. Physicians in this system may order a large palette of diagnostic tests for patients with rare diseases and unusual presentations. Furthermore, a significant number of physicians received some of their education or training in regions with a higher incidence of Lyme disease. Both factors may inflate the number of low-likelihood Lyme disease tests compared with other types of clinical settings. Importantly, the health system serves rural, suburban, and urban communities including patients presenting to a wide variety of specialty and primary care practices. Finally, our study had a small sample size and was conducted at a single center, making its generalizability uncertain.

The lack of a reliable reference standard test creates a significant challenge in clinical Lyme disease research. True-negative tests are impossible to verify, and true-positives can usually be defined only by the presence of a compatible illness. Although certain tests, such as appropriately performed culture and polymerase chain reaction, may provide more direct evidence of infection, the combination of cost, invasiveness, and lack of sensitivity exclude them from typical clinical practice. In this study, however, for all patients with positive results of 2-tiered Lyme disease serology, we believe that chart documentation sufficed for us to discriminate likely from unlikely Lyme disease.

In summary, we have described a patient cohort in which the positive predictive value of Lyme disease serologic tests is extremely low. In our study population, Lyme disease testing had an 80% rate of false-positives, which puts patients with a positive test result at risk of incorrect Lyme disease diagnoses and adverse drug reactions from inappropriate treatment. In low-transmission settings, a positive Lyme disease test result may be incapable of ruling in Lyme disease with statistical confidence, even when a compatible clinical syndrome is present.

Our findings have important implications for clinicians and public health workers in North Carolina and epidemiologically similar regions. First, clinicians must critically consider a patient's risk factors, especially recent exposure to *Ixodes* tick

habitats in regions with known Lyme disease transmission, when deciding whether to obtain (and how to interpret) serologic testing for Lyme disease. Second, physicians in nonendemic areas must carefully consider whether a positive Lyme disease test result is authentic, being careful not to miss alternative diagnoses and to counsel the patient accordingly. Finally, Lyme disease surveillance relies on the results of 2-tiered testing, including automated reporting based solely on laboratory results. This is likely to produce a high proportion of false-positives in low-transmission areas, creating further uncertainty as to the burden and distribution of this disease.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Disclaimer. The contents of this article are solely the responsibility of the authors and do not necessarily represent the official view of the National Institutes of Health (NIH).

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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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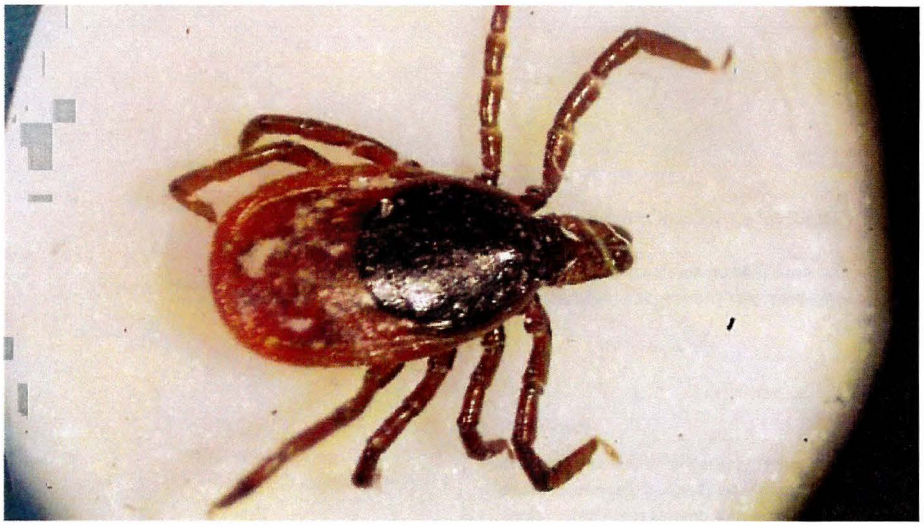
Lyme disease tests: CMAJ warns of false-positives from some U.S. labs



This March 2002 file photo shows a deer tick under a microscope in the entomology lab at the University of Rhode Island in South Kingstown, R.I. (AP Photo/Victoria Arocho)

CTVNews.ca Staff
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A commentary published in the Canadian Medical Association Journal is making the case against seeking testing for Lyme disease at some U.S. commercial labs.

In the commentary, published Monday, the authors remind readers that specialty commercial labs in the U.S. do not provide a more sensitive test for Lyme disease than what's available from public health providers.

What's more, there is a high rate of false-positive test results associated with these specialty laboratories, the commentary warns.

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Debate over Lyme disease persists as search for an accurate test continues

Still some patients who receive a negative diagnosis in Canada, seek a "second opinion" with a commercial U.S. lab, the commentary says.

"Patients with chronic subjective symptoms without a diagnosis can be vulnerable and desperate for an answer as to the cause of their illness," commentary co-author Dr. Dan Gregson, from the University of Calgary, writes.

"Giving them a false diagnosis based on flawed testing is misleading."

Lyme disease is an infectious disease caused by a strain of bacteria that's typically carried by ticks.

Symptoms of Lyme disease include fatigue, fever, the development of a rash, spasms, and swollen lymph nodes. In most cases, the disease can be treated with antibiotics.

Many Canadians with non-specific symptoms such as joint pain and fatigue may turn to commercial U.S. labs because they suspect they may have Lyme disease. However, many of these labs use only a single test that relies on non-evidence based interpretation, like the Western blot, the authors say.

"A positive test result that relies solely on Western blot testing is most likely a false-positive," the commentary says.

In one recent study, cited in the commentary, false-positive results were found in three of the four specialty U.S. labs studied, with one of the labs having a false-positive rate of more than 50 per cent.

Canada's National Microbiology Laboratory uses testing guidelines from the U.S. Centers for Disease Control and Prevention, which includes two-tier testing algorithms.

"The serologic diagnosis of Lyme disease in Canada is best done using standard laboratory protocols as implemented by the National Microbiology Laboratory of Canada using criteria recommended by the Centers for Diseae Control and Prevention," the authors conclude.

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LymeSavy 1 hour ago

"Giving them a false diagnosis based on flawed testing is misleading." ... THIS is what our current Canadian (CDC approved) testing methods do.
Everyone knows that a Lyme disease diagnose is to be clinical not based on serological testing.
We need our doctors, across the county, educated on tick borne illnesses! Thousands of lives depend on it.

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SteveWpg 10 minutes ago

This will feed the Lyme conspiracy theorists even more. It seems that Lyme is the default self-diagnosis, when mysterious ailments can't otherwise be explained. The problem is that the self-diagnosers also buy into the notion that a very aggressive (and expensive) series of antibiotic treatments is the only way to remedy themselves.

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