Performance of United States Serologic Assays in the Diagnosis of Lyme Borreliosis Acquired in Europe

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(See the Editorial Commentary by Schoen on pages 341–3.)

Background. Physicians in the United States sometimes need to evaluate a patient for suspected Lyme borreliosis (LB) who may have acquired the infection in Europe. Using serum samples from European LB patients, we compared the performance of European and US serodiagnostic tests, including newer-generation assays containing Vmp-like sequence, expressed or its C6 peptide.

Methods. The sensitivity of each assay was determined using 64 serum samples from LB patients with early or late disease manifestations who acquired the infection in Europe. Specificity was measured using 100 sera from healthy subjects from a nonendemic area.

Results. For the detection of European-acquired infection, conventional 2-tiered testing (enzyme-linked immunosorbent assay [ELISA] followed by immunoblotting) using US assays had an overall sensitivity and specificity of 52% and 100%, compared with 81% ($P = .0007$) and 99% ($P = 1.00$) using analogous European tests. The sensitivity of a US C6 ELISA used as a stand-alone test (88% overall) was statistically comparable to that of conventional 2-tiered testing using European tests ($P = .47$) and was 100% specific. Similarly, an alternative 2-tiered algorithm using a standard US ELISA followed by a C6 ELISA was comparably sensitive (84% overall) compared with conventional 2-tiered testing using European assays ($P = .82$), and specificity remained 100%.

Conclusions. European assays outperformed analogous US assays in a conventional 2-tiered testing algorithm. However, a C6 ELISA used as a stand-alone test or in the second tier of a 2-tiered algorithm performed comparably to conventional 2-tiered testing using European assays, and can be used for evaluation of any patient, regardless of travel history.

Keywords. Lyme; *Borrelia burgdorferi*; VlsE; C6; diagnosis.

Lyme borreliosis (LB) is endemic in expanding regions of North America and Europe. In the United States, the infection is caused by a single *Borrelia* species, *B. burgdorferi* sensu stricto (s.s.) [1]. Although there is genetic and antigenic diversity among North American strains

of *B. burgdorferi* s.s. [2, 3], these strains share key immunodominant antigens [4]. Therefore, although US serologic assays are typically manufactured from lysates of the original *B. burgdorferi* s.s. isolate, called strain B31, any *B. burgdorferi* s.s. strain has usually worked well for serologic assays [4–6].

In contrast, European LB is caused by several species within the *B. burgdorferi* sensu lato (s.l.) complex, primarily *Borrelia afzelii* and *Borrelia garinii*, and less often, *B. burgdorferi* s.s. [7]. Furthermore, there is substantial heterogeneity among strains within each species, including the antigens most relevant to serodiagnosis [7, 8]. Consequently, for European immunoblot assays, the choice of species and strain is thought to be

important for optimal performance [9, 10], although it may be less important for enzyme-linked immunosorbent assays (ELISAs) [11]. Therefore, European immunoblots are prepared from spirochetal lysates and/or purified antigens from various *B. burgdorferi* s.l. strains and species, reflective of the region in which the test is used. In addition, interpretive criteria for immunoblots have been defined based on the species and strains selected for a particular assay [12–14], precluding universal interpretive guidelines such as those recommended by the Centers for Disease Control and Prevention (CDC) for use in the United States [15].

Americans who travel to Europe may acquire the infection there, but may return to the United States for evaluation. The question then arises: Can the infection acquired in Europe be detected with standard 2-tiered serologic testing, using assays designed for use in the United States? In such cases, diagnostic failures have been reported using US assays [16]. However, the performance of US and European assays has not been compared systematically using sera from patients who acquired the infection in Europe. Moreover, newer-generation assays are now available in the United States that incorporate the Vmp-like sequence, expressed (VlsE) protein [17], or an immunodominant, largely conserved 25-mer oligopeptide (C6 peptide) corresponding to the sixth invariable region within VlsE [18, 19]. These antigen targets have been shown to perform well in identifying European LB cases [20–23].

We compared here the performance of widely used, commercially available European and US serologic assays for LB, including those containing recombinant VlsE (rVlsE) or its C6 peptide, using serum specimens collected from patients who acquired the infection in the southern part of central Europe. We found that conventional 2-tiered testing using US assays was inferior to 2-tiered testing using analogous European assays. However, VlsE or C6 peptide assays used as a stand-alone test or in the second tier of a 2-tiered algorithm performed comparably to conventional 2-tiered testing using European assays.

PATIENTS AND METHODS

Patient Samples

All patients were evaluated at the Lyme Borreliosis Outpatient Clinic at University Medical Center Ljubljana (Slovenia) between January 1999 and October 2009, and met the European criteria for LB [24]. The study approach was approved by the Medical Ethics Committee of the Republic of Slovenia's Ministry of Health.

Patients with erythema migrans (EM) had a typical, solitary skin lesion, without other organ involvement or past history of LB, and 15 of the 20 patients in this group had positive cultures (9 with *B. afzelii*, 6 with *B. garinii*) from skin biopsy samples.

In addition to compatible clinical findings, all patients with Lyme neuroborreliosis (LNB) had a cerebrospinal fluid pleocytosis as well as concomitant EM, isolation of *B. burgdorferi* s.l. spriochetes from cerebrospinal fluid or demonstration of intrathecal synthesis of *Borrelia*-specific antibodies. Patients with Lyme arthritis (LA) had swelling in 1 or more large joints, and *Borrelia*-specific antibodies in serum, without an alternative explanation for the arthritis. Patients with acrodermatitis chronica atrophicans (ACA) had a characteristic clinical picture, supportive histologic findings, and high serum immunoglobulin G (IgG) antibody levels against specific *Borrelia* antigens. All patient serum samples were obtained prior to antibiotic treatment, aliquoted, and stored frozen at −80°C until analysis. These samples were used in previous studies [25–27]. Control sera were collected in 2007 from consenting healthy blood donors in New Zealand, a nonendemic region for LB; these samples were also included in previous studies [28, 29].

Serologic Testing

Serum samples were tested using European and US first-tier ELISAs and second-tier immunoblot assays (Table 1). The European ELISAs included the Enzygnost immunoglobulin M (IgM) and IgG assays, and the American ELISAs included the Wampole IgG/IgM assay and the VlsE C6 peptide assay. Both the IgM and IgG immunoblots, designed for use in the United States or Europe, were manufactured by Viramed Biotech AG. Standard CDC criteria as well as hybrid IgG + VlsE criteria were used to assess US immunoblots, and "MiQ 12 plus VlsE" criteria were used to assess European immunoblots (Table 2). All testing was performed according to the manufacturers' instructions. The immunoblots were read with the aid of densitometry; a band was scored as present if its intensity was ≥90% of the cutoff control band's intensity (except for the p41 band on the European IgM immunoblots, which was required to be ≥200% of the cutoff band's intensity).

Sera from the New Zealand control subjects had been tested previously using the VIDAS Lyme IgG/IgM assay (bioMérieux SA; Marcy-l'Etoile, France), a polyvalent, whole-cell sonicate (WCS) enzyme-linked fluorescent assay prepared from *B. burgdorferi* s.s. strain B31, and also using the same VlsE C6 peptide ELISA and US immunoblots employed in the present study [28, 29]. Because the VIDAS ELISA gives very similar results as the Wampole IgG/M ELISA, and because these serum samples were limited, the previous results were used again here. However, the control serum samples were tested in the present study using the European serologic assays described in Table 1.

Statistical Analysis

Differences between proportions were considered significant if the 2-tailed *P* value was ≤.05, as determined using Fisher's exact test.

Table 1. European and US Serodiagnostic Tests Evaluated in This Study

Test	Source	Test Name	Manufacturer	Antibody Isotype Detected	Antigen Source
ELISAs	European	Enzygnost Borreliosis	Siemens/Dade Behring, Marburg, Germany	IgM	Lysate of *Borrelia afzelii* strain PKo
		Enzygnost Lyme Link VlsE/IgG	Siemens/Dade Behring, Marburg, Germany	IgG	Lysate of *B. afzelii* strain PKo, plus rVlsE derived from *B. afzelii*, *Borrelia garinii*, and *Borrelia burgdorferi* s.s.
	US	Wampole *B. burgdorferi* IgG/IgM ELISA II	Alere Inc, Waltham, MA	IgM, IgG	Lysate of *B. burgdorferi* s.s. strain B31
		C6 *B. burgdorferi*	Immunetics, Inc, Boston, MA	IgM, IgG	25-mer amino acid peptide corresponding to IR_6 of *B. burgdorferi* s.s. strain B31
Immunoblots	European	*Borrelia* MiQ + VlsE IgM test kit	Viramed Biotech AG, Planegg, Germany	IgM	Extracts of *B. afzelii* strain PKo and *B. burgdorferi* strain B31 combined, supplemented by direct application of rVlsE derived from *B. burgdorferi* s.s. strain B31
		Borrelia MiQ + VlsE IgG test kit	Viramed Biotech AG, Planegg, Germany	IgG	Extracts of *B. afzelii* strain PKo and *B. burgdorferi* s.s. strain B31 combined, supplemented by direct application of rVlsE derived from *B. burgdorferi* s.s. strain B31
	US	*Borrelia* B31 ViraBlot IgM test kit	Viramed Biotech AG, Planegg, Germany	IgM	Extract of *B. burgdorferi* s.s. strain B31
		Borrelia B31 plus VlsE ViraBlot IgG test kit	Viramed Biotech AG, Planegg, Germany	IgG	Extract of *B. burgdorferi* s.s. strain B31, supplemented by direct application of rVlsE derived from *B. burgdorferi* s.s. strain B31

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IgM, immunoglobulin M; rVlsE, recombinant VlsE; s.s., sensu stricto; VlsE, Vmp-like sequence, expressed.

RESULTS

First-Tier Tests

Using serum samples from 64 patients who acquired LB in Slovenia, the sensitivity of a US first-tier WCS IgM/IgG ELISA was 70% in patients with EM, 87% in patients with acute LNB, 93% in patients with LA, and 100% in patients with ACA, a late disease manifestation (Table 3). Similar results were obtained with a European first-tier ELISA test system, which contains not only WCS from a European strain, but also rVlsE, and with a US C6 peptide ELISA. Using serum samples from 100 healthy control subjects living in a nonendemic area, the specificity of these US and European assays was also similar. The European WCS IgM ELISA produced 2 false-positive results among 100 control sera, and the European WCS IgG/rVlsE ELISA also produced 2 false-positive results, for a combined specificity of 96%. The US polyvalent WCS ELISA produced 3 false-positive results (97% specificity), and the US C6 peptide ELISA produced no false-positive results (100% specificity). Thus, there were no significant differences in sensitivity or specificity among these assays in the detection of European-acquired LB.

Second-Tier Tests

Unlike with first-tier assays, there were significant differences in the sensitivity and specificity of second-tier European IgM and IgG immunoblot assays when compared with US IgM and IgG immunoblot assays (Table 4). Using European immunoblots, the sensitivity was 55% in patients with EM and 87% in patients with LNB, whereas using US immunoblots, the percentages were 20% ($P = .05$) and 53% ($P = .11$), respectively. In the later stages of infection, however, sensitivity was similar: 93% of LA patients and 100% of ACA patients had a positive European immunoblot, compared with 73% ($P = .33$) and 100%

Table 2. Interpretive Criteria Applied to European and US Immunoblot Assays

Immunoblot	Criteria for a Positive Result
European IgM	≥1 of the following 5 bands must be present: VlsE, p39, p41 (strong intensity), OspC, DbpA (Osp17) [40]
European IgG	≥2 of the following 10 bands must be present: VlsE, p83/100, p58, p43, p39, p30, OspC, p21, Osp17, p14 [40]
US IgM	Conventional CDC criteria [15]: ≥2 of the following 3 bands must be present: 24 kDa (OspC), 39 kDa (BmpA), and 41 kDa (Fla)
	Hybrid IgG + VlsE criteria [28]: an IgM immunoblot is not used; the criteria rely solely on an IgG immunoblot that has been modified by the addition of rVlsE in a specified location.
US IgG	Conventional CDC criteria: ≥5 of the following 10 bands must be present: 18 kDa, 21 kDa (OspC), 28 kDa, 30 kDa, 39 kDa (BmpA), 41 kDa (Fla), 45 kDa, 58 kDa (not GroEL), 66 kDa, and 93 kDa [15]
	Hybrid IgG + VlsE criteria [28]: When early infection is suspected (EM, acute carditis, or acute LNB), the rVlsE band alone is scored, and the IgG immunoblot is considered positive if the rVlsE band is present. When late infection is suspected (LA or ACA), 11 bands on the IgG immunoblot are scored (the 10 bands included in the CDC IgG criteria listed above, plus the rVlsE band), and the immunoblot is considered positive if ≥5 of the 11 bands are present.

Abbreviations: ACA, acrodermatitis chronica atrophicans; CDC, Centers for Disease Control and Prevention; EM, erythema migrans; IgG, immunoglobulin G; IgM, immunoglobulin M; LA, Lyme arthritis; LNB, Lyme neuroborreliosis; rVlsE, recombinant VlsE; VlsE, Vmp-like sequence, expressed.

($P = 1.00$), respectively, using US immunoblots. When all patients with LB were considered together, without regard to

disease stage, 81% had a positive European IgM or IgG immunoblot, compared with 58% using US immunoblots ($P = .007$). Among 100 healthy control subjects, the European IgM immunoblots produced 9 false-positive results (91% specificity), whereas the US IgM immunoblots yielded no false-positive results (100% specificity; $P = .003$). Both the European and US IgG immunoblot methods were 100% specific. Thus, the significantly greater sensitivity achieved with European immunoblots was counterbalanced by significantly reduced specificity.

Conventional 2-Tiered Testing

Results obtained using European or US assays were compared after applying a conventional 2-tiered testing algorithm, in which the first step is either a polyvalent WCS ELISA or separate IgM and IgG WCS ELISAs, followed by IgM and IgG immunoblots (Table 5). For the European immunoblots, "MiQ 12 plus VlsE" interpretive criteria were applied, as described in Table 2, because these criteria are recommended by the assay manufacturer. For the US immunoblots, standard CDC interpretive criteria were used (Table 2).

Using US assays in a conventional 2-tiered algorithm, sensitivity in the detection of European-acquired infection was 20% in patients with EM, 40% in those with LNB, 60% in those with LA, and 100% in those with ACA. In comparison, sensitivity using European tests was significantly higher in patients with EM (55%, $P = .05$) and LNB (87%, $P = .02$), and was also higher in patients with LA (93%), a difference of possible significance ($P = .08$). Equivalent sensitivity was seen in those with ACA (100%, $P = 1.00$). When all patients with LB were considered together, conventional 2-tiered testing using US assays was 52% sensitive compared with 81% using European assays ($P = .0007$). Among healthy control subjects, conventional 2-tiered testing with US methods yielded 100% specificity, whereas

Table 3. Performance of European and US First-Tier Enzyme-Linked Immunosorbent Assays

	No. Positive (%)				
	European WCS IgM	European WCS/rVlsE IgG	European IgM or IgG	US WCS IgM/IgG	US C6 Peptide
Patients with Lyme borreliosis acquired in Europe (n = 64)	39 (61)	55 (86)	58 (91)	55 (86) [0.58]	56 (88) [.78]
Erythema migrans (n = 20)	8 (40)	13 (65)	15 (75)	14 (70) [1.00]	14 (70) [1.00]
Lyme neuroborreliosis (n = 15)	12 (80)	13 (87)	14 (93)	13 (87) [1.00]	13 (87) [1.00]
Lyme arthritis (n = 15)	9 (60)	15 (100)	15 (100)	14 (93) [1.00]	15 (100) [1.00]
Acrodermatitis chronica atrophicans (n = 14)	10 (71)	14 (100)	14 (100)	14 (100) [1.00]	14 (100) [1.00]
Healthy control subjects from nonendemic area (n = 100)	2 (2)	2 (2)	4 (4)	3 (3) [1.00]	0 (0) [0.12]

Bracketed numbers represent the P values for the comparison with combined results obtained using European IgM and IgG enzyme-linked immunosorbent assays.

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; rVlsE, recombinant Vmp-like sequence, expressed; WCS, whole-cell sonicate.

Table 4. Performance of European and US Second-Tier Immunoblot Assays

	No. Positive (%)					
	European IgM IB[a]	European IgG IB[a]	European IgM or IgG IB[a]	US IgM IB[b]	US IgG IB[b]	US IgM or IgG IB[b]
Patients with Lyme borreliosis acquired in Europe (n = 64)	34 (53)	43 (67)	52 (81)	16 (25)	32 (50)	37 (58) [.007]
Erythema migrans (n = 20)	7 (35)	7 (35)	11 (55)	2 (10)	2 (10)	4 (20) [.05]
Lyme neuroborreliosis (n = 15)	12 (80)	9 (60)	13 (87)	6 (40)	6 (40)	8 (53) [.11]
Lyme arthritis (n = 15)	10 (67)	13 (87)	14 (93)	4 (27)	10 (67)	11 (73) [0.33]
Acrodermatitis chronica atrophicans (n = 14)	5 (36)	14 (100)	14 (100)	4 (29)	14 (100)	14 (100) [1.00]
Healthy control subjects from nonendemic area (n = 100)	9 (9)	0 (0)	9 (9)	0 (0)	0 (0)	0 (0) [.003]

Bracketed numbers represent the P values for the comparison with combined results obtained using European IgM and IgG immunoblots.

Abbreviations: IB, immunoblot; IgG, immunoglobulin G; IgM, immunoglobulin M.

[a] Interpreted according to European "MiQ 12 plus VlsE" criteria (Table 2) [40].

[b] Interpreted according to standard Centers for Disease Control and Prevention criteria (Table 2) [15], except that duration of illness was not considered.

Table 5. Performance of Conventional and Alternative 2-Tiered Testing Algorithms Using European or US Serodiagnostic Assays

	No. Positive (%)					
	Conventional 2-Tier Using European Tests[a]	Conventional 2-Tier Using US Tests[b]	C6 ELISA Followed by US Immunoblots[c]	US Tests and Hybrid IgG + VlsE Criteria[d]	US 2-EIA Algorithm (WCS + C6)[e]	C6 Peptide ELISA Alone
Patients with Lyme borreliosis acquired in Europe (n = 64)	52 (81)	33 (52) [.0007]	34 (53) [.001]	47 (73) [.40]	54 (84) [.82]	56 (88) [.47]
Erythema migrans (n = 20)	11 (55)	4 (20) [.05]	4 (20) [.05]	11 (55) [1.00]	13 (65) [.75]	14 (70) [.51]
Lyme neuroborreliosis (n = 15)	13 (87)	6 (40) [.02]	6 (40) [.02]	13 (87) [1.00]	13 (87) [1.00]	13 (87) [1.00]
Lyme arthritis (n = 15)	14 (93)	9 (60) [.08]	10 (67) [.17]	9 (60) [.08]	14 (93) [1.00]	15 (100) [1.00]
Acrodermatitis chronica atrophicans (n = 14)	14 (100)	14 (100) [1.00]	14 (100) [1.00]	14 (100) [1.00]	14 (100) [1.00]	14 (100) [1.00]
Healthy control subjects from nonendemic area (n = 100)	1 (1)	0 (0) [1.00]	0 (0) [1.00]	0 (0) [1.00]	0 (0) [1.00]	0 (0) [1.00]

Bracketed numbers represent the P values for the comparison with results obtained by 2-tiered testing using tests designed for use in Europe.

Abbreviations: EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; VlsE, Vmp-like sequence, expressed; WCS, whole-cell sonicate.

[a] The values represent the number of serum samples that were positive or equivocal by European WCS immunoglobulin M(IgM) or WCS/recombinant Vmp-like sequence (rVlsE) IgG ELISA and positive by either IgM or IgG European immunoblots, interpreted according to European "MiQ 12 plus VlsE" criteria (Table 2).

[b] The values represent the number of serum samples that were positive or equivocal by US WCS polyvalent (IgM/IgG) ELISA and positive by either IgM or IgG US immunoblots, interpreted according to standard Centers for Disease Control and Prevention (CDC) criteria (Table 2). IgM criteria were not used if the duration of symptoms was >1 month at the time of specimen collection.

[c] The values represent the number of serum samples that were positive or equivocal by the US C6 peptide ELISA and positive by either IgM or IgG US immunoblots, interpreted according to standard CDC criteria. IgM criteria were not used if the duration of symptoms was >1 month at the time of specimen collection.

[d] For patients with erythema migrans or Lyme neuroborreliosis, and the control subjects, the values in this column represent the number of serum samples that were positive or equivocal by US WCS polyvalent ELISA and positive for the rVlsE band on US IgG immunoblots. For patients with Lyme arthritis or acrodermatitis chronica atrophicans, the values represent the number of serum samples that were positive or equivocal by US WCS polyvalent ELISA and that produced ≥5 of 11 specific IgG bands on US IgG immunoblots (10 bands described in standard CDC criteria, plus the rVlsE band).

[e] The values represent the number of serum samples that were positive or equivocal by US WCS polyvalent ELISA and positive or equivocal by US C6 peptide ELISA.

European methods produced 1 false-positive result (99% specificity; $P = 1.00$).

Alternative Testing Algorithms

A VlsE C6 peptide ELISA was assessed as a stand-alone test, and 3 alternative 2-tiered testing algorithms were also evaluated, each of which incorporate an element to detect either anti-IR_6 or anti-VlsE antibodies (Table 5). First, 2-tiered testing using a US C6 peptide ELISA followed by US IgM and IgG immunoblots was assessed, with the immunoblots interpreted according to standard CDC criteria. Overall, this approach was significantly less sensitive (53% overall) than conventional 2-tiered testing using European assays (81% overall, $P = .001$); specificity was 100% and 99%, respectively ($P = 1.00$). With this approach, the advantages of the C6 peptide assay were limited by the conventional WCS immunoblot as a second-tier test. The sensitivity could be improved using an alternative 2-tiered approach in which a US WCS ELISA was followed by an immunoblot with a VlsE stripe, interpreted with previously published hybrid criteria [28] (Table 2). This approach yielded an overall sensitivity of 73%; this was still less sensitive than conventional 2-tiered testing using European assays (81%, $P = .40$), but the difference was not statistically significant; specificity remained 100%.

Two other approaches gave better results—a C6 peptide ELISA alone or a 2–enzyme immunoassay (2-EIA) approach using a US WCS polyvalent ELISA followed by a C6 ELISA (Table 5). By itself, the C6 ELISA had a sensitivity of 70% in patients with EM, 87% in those with LNB, 100% in those with LA, and 100% in those with ACA. Similarly, the 2-EIA algorithm had a sensitivity of 65% in patients with EM, 87% in those with LNB, 93% in those with LA, and 100% in those with ACA. When applied to all 64 LB patients, European assays used in a conventional 2-tiered algorithm had a sensitivity of 81% compared with 88% using the C6 assay alone ($P = .47$) and 84% using the US 2-EIA algorithm ($P = .82$). Both the C6 ELISA and the US 2-EIA algorithm were 100% specific, compared with 99% specificity by 2-tiered testing using European assays ($P = 1.00$).

DISCUSSION

In patients who acquire LB in Europe, the *B. burgdorferi* s.l. species or strain used in antigen preparation is thought to make a difference in serologic reactivity [7, 9, 10, 30, 31]. Therefore, tests designed for use in Europe are typically prepared from a broader representation of species and strains than those used in the United States, and use individualized algorithms and scoring criteria for interpretation. For this reason, it is more difficult to know which European tests to compare with US tests. For example, it is more common in Europe to employ ELISAs

as a stand-alone test, whereas the standard approach in the United States is to use ELISAs as first-step screening tests in a 2-tiered algorithm.

In this study, conventional 2-tiered testing using a US polyvalent WCS ELISA followed by US IgM and IgG immunoblots, interpreted according to standard CDC criteria, was significantly less sensitive compared with 2-tiered testing using analogous European serologic assays, when applied to sera from European LB patients. Several factors may explain this difference. First, as expected, the European patients with culture-positive EM were shown to have infection with *B. afzelii* or *B. garinii* and not *B. burgdorferi* s.s, as in the United States. Second, the European first-tier ELISA and second-tier immunoblots were supplemented by the addition of rVlsE. When the European immunoblots were scored without the inclusion of the VlsE band in the interpretive criteria, sensitivity was modestly reduced in the early stages of infection (data not shown).

However, we think that the most important difference was the interpretative criteria. With the European criteria, a positive immunoblot requires only ≥ 1 of 5 IgM bands or ≥ 2 of 10 IgG bands, whereas in the United States, CDC criteria require ≥ 2 of 3 IgM bands or ≥ 5 of 10 IgG bands. The less stringent criteria used in Europe tend to bolster sensitivity at the expense of specificity. As was seen here, the sensitivity of US immunoblots was limited primarily in early disease. This limitation could be alleviated by using an IgG immunoblot supplemented with a VlsE stripe, interpreted with modified "hybrid" criteria [28]. With this approach, an IgM immunoblot was not performed; only the VlsE band was scored in early disease, whereas 5 of 11 bands were scored in late disease, 1 of which could be VlsE [28]. In early disease, this alternative 2-tiered algorithm was significantly more sensitive than conventional US 2-tiered testing and equivalent to that of European 2-tiered testing.

Importantly, 2 alternative approaches—a US C6 peptide ELISA as a stand-alone test and a 2-EIA approach in which a US WCS ELISA was followed by the C6 ELISA—gave very similar results that were significantly better than those produced by conventional 2-tiered testing using US assays and slightly (but not significantly) better than those produced by conventional 2-tiered testing using European assays. Both of these alternative approaches employ Food and Drug Administration–cleared ELISAs designed for use in the United States, and are readily available to clinicians in North America. Thus, serologic assays employing *B. burgdorferi* s.s. VlsE or its C6 peptide are able to detect antibody responses elicited by other *B. burgdorferi* s.l. species. This comports with previous animal and human studies showing that the IR_6 region of VlsE is antigenically conserved across Lyme-related genospecies [32] despite the existence of slight interspecies sequence heterogeneity within IR_6 [33] and despite differences in reactivity among European LB patients with assays using rVlsE or C6 peptide

derived from different *B. burgdorferi* s.l. species [30, 33–35]. In addition, an advantage of the C6 peptide ELISA is that an IgG antibody response to this peptide develops early in the disease and typically persists through later stages of the infection.

Compared with the C6 peptide ELISA as a stand-alone test, the combination of the WCS ELISA and the C6 peptide ELISA slightly—but not significantly—reduced the sensitivity of the latter test, an effect that was also shown in a previous study on US LB patients [29]. However, we think that the more important difference is in specificity. Although the C6-peptide ELISA employed in this study was 100% specific using 100 serum samples from healthy control subjects from New Zealand, a larger study showed that about 1 in 50 to 1 in 100 healthy control subjects had false-positive results with the C6 ELISA alone, whereas the 2-EIA approach remained 100% specific [29]. This difference in specificity translates into large differences in positive predictive value, because the prevalence of LB in tested populations is usually low, even in areas of endemicity. In the United States, where at least 3.4 million Lyme disease tests are performed yearly [36], this difference would lead to an additional 37 000 false-positive results per year, which exceeds the number of LB cases reported annually [37]. For this reason, we favor the 2-EIA approach.

The European IgG ELISA used in this study contained not only WCS sonicate from a European *B. burgdorferi* s.l. species, but also rVlsE derived from multiple strains. Although this test had a very similar sensitivity compared with the US 2-EIA approach, which employs WCS and the VlsE C6 peptide as separate tests, the specificity of the European IgG ELISA alone was not quite as good (100% vs 98%). Thus, by combining in the same test rVlsE and WCS, which contains little if any VlsE, one loses the advantage in specificity gained by doing the tests separately.

Several points require explanation. First, the frequency of seropositivity in this study using first-tier ELISAs was higher in European patients with EM (approximately 70%) than has been reported previously in US patients [38, 39]. We think this is explained by the duration of EM prior to evaluation, which was a mean of 5 days in a prior US study [38] and 14 days in the present study ($P = .003$). Second, European patients in this study came from southern Central Europe, so it is uncertain whether the findings would apply to patients who acquire the infection elsewhere in Europe. Although regional spirochetal strain variation is a potential factor in test results, this is primarily a problem with immunoblotting and less so with first-tier tests [11]. Thus, it is likely that our findings would be applicable to other European regions.

In summary, conventional 2-tiered serologic testing using assays designed for use in the United States was significantly less sensitive compared with 2-tiered testing using European assays in patients with LB acquired in Europe. However,

alternative approaches that include VlsE or its C6 peptide as a stand-alone test or in the second-tier of a 2-tiered algorithm were comparable with conventional 2-tiered assays designed for use in Europe. Moreover, it has also been demonstrated that the 2-EIA algorithm is significantly more sensitive when compared to conventional US 2-tiered testing for patients who have acquired the infection in the United States, and is equally specific [29]. Therefore, the 2-EIA algorithm can be applied to any patient being evaluated for LB, regardless of travel history.

Notes

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