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Evaluation of bioMérieux's Dissociated Vidas Lyme IgM II and IgG II as a First-Tier Diagnostic Assay for Lyme Disease

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ABSTRACT The recommended laboratory diagnostic approach for Lyme disease is a standard two-tiered testing (STTT) algorithm where the first tier is typically an enzyme immunoassay (EIA) that if positive or equivocal is reflexed to Western immunoblotting as the second tier. bioMérieux manufactures one of the most commonly used first-tier EIAs in the United States, the combined IgM/IgG Vidas test (LYT). Recently, bioMérieux launched its dissociated first-tier tests, the Vidas Lyme IgM II (LYM) and IgG II (LYG) EIAs, which use purified recombinant test antigens and a different algorithm than STTT. The dissociated LYM/LYG EIAs were evaluated against the combined LYT EIA using samples from 471 well-characterized Lyme patients and controls. Statistical analyses were conducted to assess the performance of these EIAs as first-tier tests and when used in two-tiered algorithms, including a modified two-tiered testing (MTTT) approach where the second-tier test was a C6 EIA. Similar sensitivities and specificities were obtained for the two testing strategies (LYT versus LYM/LYG) when used as first-tier tests (sensitivity, 83 to 85%; specificity, 85 to 88%) with an observed agreement of 80%. Sensitivities of 68 to 69% and 76 to 77% and specificities of 97% and 98 to 99% resulted when the two EIA strategies were followed by Western immunoblotting and when used in an MTTT, respectively. The MTTT approach resulted in significantly higher sensitivities than did STTT. Overall, the LYM/LYG EIAs performed equivalently to the LYT EIA in test-to-test comparisons or as first-tier assays in STTT or MTTT with few exceptions.

KEYWORDS Lyme disease, Vidas, serology, *Borrelia burgdorferi*

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Laboratory diagnosis of Lyme disease in the United States is currently based on the detection of serologic responses to *Borrelia burgdorferi*, the most common cause of Lyme disease. The Centers for Disease Control and Prevention (CDC) recommends a standardized two-tiered testing (STTT) algorithm that utilizes a first-tier immunofluorescence assay (IFA) or enzyme immunoassay (EIA) that if positive or equivocal is followed by a second-tier assay consisting of IgM and/or IgG Western immunoblotting (1). Both assays must be positive or equivocal for laboratory support of a Lyme disease diagnosis. There are numerous first-tier assays that have been cleared by the Food and Drug Administration (FDA) for use as first-tier tests since 1995. These are primarily EIAs that use either *B. burgdorferi* whole-cell sonicate (WCS), whole proteins, or a single peptide (2, 3). Among these EIAs is bioMérieux's polyvalent Vidas LYT, which detects IgM and IgG antibodies to *B. burgdorferi* in human serum. This assay uses a WCS preparation from *B. burgdorferi* strain B31 and has been used extensively in clinical laboratories that test for Lyme disease. Additionally, this assay is fully automated and has been shown to produce a higher sensitivity than other commercially available EIAs (4, 5). However, the LYT assay yields a lower specificity than some of the other commercially available EIAs and does not differentiate between IgM and IgG antibody reactivity (6). More recently, bioMérieux developed a strategy for first-tier Lyme disease

TABLE 1 Reactivity of first-tier bioMérieux EIAs and the C6 EIA

Samples (no.)	No. positive (%)					
				Vidas LYM/LYG		
	Vidas LYT[a]	Vidas LYM[b]	Vidas LYG	No duration of illness considered for LYM testing	Duration of illness was considered for LYM testing	C6[a]
Lyme disease (124)						
Early Lyme disease with EM						
Acute (40)	27 (68)	24 (60)	20 (50)	27 (68)	27 (68)	23 (58)
Convalescent (38)	34 (89)	30 (79)	28 (74)	30 (79)	30 (79)	32 (84)
Lyme neuroborreliosis or Lyme carditis						
Lyme neuroborreliosis (10)	9 (90)	10 (100)	9 (90)	10 (100)	10 (100)	10 (100)
Lyme carditis (7)	7 (100)	5 (71)	6 (86)	7 (100)	7 (100)	6 (86)
Late Lyme disease, Lyme arthritis (29)	29 (100)	19 (63)	29 (100)	29 (100)	29 (100)	29 (100)
All Lyme disease	106 (85)	88 (71)	92 (74)	103 (83)	103 (83)	100 (81)
Negative controls (347)						
Other diseases (144)						
Fibromyalgia (31)	0 (0)	3 (10)	2 (6)	5 (16)	2 (6)	0 (0)
Severe periodontitis (20)	0 (0)	1 (5)	0 (0)	1 (5)	0 (0)	0 (0)
Rheumatoid arthritis (21)	2 (10)	4 (19)	1 (5)	5 (24)	1 (5)	0 (0)
Syphilis (20)	17 (85)	4 (20)	2 (10)	6 (30)	2 (10)	2 (10)
Multiple sclerosis (22)	4 (18)	1 (5)	0 (0)	1 (5)	0 (0)	1 (5)
Infectious mononucleosis (30)	16 (53)	8 (27)	0 (0)	8 (27)	8 (27)	4 (13)
All other diseases	39 (27)	21 (15)	5 (3)	26 (18)	13 (9)	7 (5)
Healthy controls (203)						
Endemic[c] (101)	9 (9)	14 (14)	3 (3)	17 (17)	17 (17)	1 (1)
Nonendemic[d] (102)	5 (5)	9 (9)	1 (1)	10 (10)	10 (10)	4 (4)
All healthy controls	14 (7)	23 (11)	4 (2)	27 (13)	27 (13)	5 (2)
All negative controls	53 (15)	44 (13)	9 (3)	53 (15)	40 (12)	12 (3)

[a]These data have been reported previously (4, 14).
[b]Positive LYM results were reported regardless of duration of illness prior to specimen collection.
[c]Endemic, samples from controls in areas where Lyme disease is endemic.
[d]Nonendemic, samples from controls in areas where Lyme disease is not endemic.

testing that separately detects IgM antibodies (LYM assay) to recombinant antigens DbpA and OspC and IgG antibodies (LYG assay) to recombinant antigens VlsE, DbpA, and OspC. Both assays contain recombinant antigens from *B. burgdorferi sensu stricto*, *Borrelia afzelii*, and *Borrelia garinii*. Additionally, all three antigens used in these assays have been shown to be immunogenic during human infection (7–13). Both assays utilize the same automated platform as the WCS (LYT EIA) assay, both use 100 μl of serum or plasma for each assay, and both are FDA cleared. The dissociated assays are run simultaneously, and corresponding immunoblot assays are run for any positive or equivocal LYM EIA and/or positive LYG EIA. Scoring of subsequent immunoblot assays is done according to the recommended guidelines (1).

In this study, serum samples from the CDC Lyme Serum Repository (LSR) were used to test the dissociated LYM and LYG EIAs, and the resulting data were compared to the results for the combined LYT EIA (4). The overall sensitivities and specificities for STTT were similar between the two testing strategies (LYT versus LYM/LYG), although differences in first-tier test results between the two were observed. A modified two-tiered (MTTT) algorithm that uses the Vidas EIAs (LYT or LYM/LYG) as the first-tier test followed by the C6 EIA as the second-tier test also gave similar sensitivities and specificities when the dissociated or combined assays were tested but resulted in significantly higher overall sensitivities than and specificities similar to those of STTT.

RESULTS

First-tier testing. The results for the two bioMérieux EIA strategies as first-tier tests for Lyme disease laboratory diagnosis are summarized in Table 1. The sensitivities obtained for acute early Lyme disease patients with erythema migrans (EM) were similar (68%) when using the combined LYT EIA and when using the dissociated LYM/LYG EIAs. The resulting sensitivity for convalescent early Lyme disease patients

TABLE 2 Agreement between the combined LYT and dissociated LYM and LYG EIAs[a]

Samples (no.)	% agreement	Kappa value[b]
Lyme disease (124)		
Early Lyme disease with EM		
Acute (40)	70	0.32 (0.01, 0.63)
Convalescent (38)	79	0.21 (0.11, 0.53)
Lyme neuroborreliosis or Lyme carditis		
Lyme neuroborreliosis (10)	90	−0.05 (−0.67, 0.57)
Lyme carditis (7)	100	
Late Lyme disease, Lyme arthritis (29)	100	NaN[c]
Negative controls (347)		
Other diseases (144)		
Fibromyalgia (31)	94	−0.03 (−0.39, 0.32)
Severe periodontitis (20)	100	NaN
Rheumatoid arthritis (21)	86	−0.08 (−0.50, 0.57)
Syphilis (20)	25	−0.50 (−0.94, −0.07)
Multiple sclerosis (22)	82	−0.10 (−0.52, 0.32)
Infectious mononucleosis (29)	53	0.03 (−0.33, 0.39)
Healthy controls (203)		
Endemic[d] (101)	80	0.12 (0.08, 0.31)
Nonendemic[d] (102)	87	0.06 (−0.13, 0.26)
All samples tested (471)	80	0.55 (0.46, 0.64)

[a]A ≤30-day duration-of-illness cutoff was considered for LYM testing.
[b]The 95% confidence intervals are indicated in parentheses.
[c]NaN, not a number; the kappa value cannot be computed because the two EIA strategies were in complete agreement.
[d]Endemic and nonendemic are as defined in the footnotes to Table 1.

with EM was higher when the LYT EIA (89% sensitivity) was used than when the LYM/LYG EIAs (79% sensitivity) were used. For other Lyme disease groups (Lyme carditis, Lyme neuroborreliosis, and Lyme arthritis), sensitivities were similar for the two EIA strategies (100% for Lyme carditis, 90 to 100% for Lyme neuroborreliosis, and 100% for Lyme arthritis). It should be noted that a small sample size was used for assessing the performance of these two assays in patients diagnosed with Lyme carditis and Lyme neuroborreliosis. When all negative controls were tested, the specificities of the two EIA strategies were the same (85% specificity) when duration of illness was not considered for LYM testing in patients with other diseases. However, when duration of illness was considered for patients with other diseases, the specificity of the dissociated assays increased to 88%. The LYT EIA had higher cross-reactivity when samples from patients with other diseases were tested (73% specificity) than the LYM/LYG EIAs (91% and 82% specificity when duration of illness was considered and not considered for LYM testing, respectively). Conversely, the dissociated LYM/LYG EIAs showed higher cross-reactivity when healthy controls were tested (87% specificity) than did the LYT EIA (93% specificity) (Table 1).

Pairwise comparisons of the two testing strategies (LYM/LYG versus LYT) showed that when all samples were tested, there was no significant difference between the two first-tier testing strategies (see Table S1 in the supplemental material). However, when samples were broken out into subgroups (Lyme disease, other diseases, and healthy controls), significant differences were observed for other diseases ($P < 0.01$) and healthy controls ($P = 0.04$). Specifically, the LYM/LYG EIAs (with duration of illness considered for LYM testing) resulted in an 18% decrease in false-positive results for other diseases compared to the LYT EIA. This increased specificity was largely due to more correct calls for syphilis and infectious mononucleosis samples and resulted in test agreements of only 25% and 53%, respectively (Table 2). For healthy controls, the LYM/LYG EIAs resulted in a 6% increase in false-positive results over the LYT EIA. The percentages of agreement between the two EIA testing strategies for healthy controls from areas where Lyme disease is endemic and areas where it is not endemic were 80% and 87%, respectively.

TABLE 3 Results of two-tiered algorithms tested

Sample (no.)	No. positive (%)				
	Two-tiered testing[a,b]			Two-EIA algorithms (MTTT)	
	LYT-ViraStripe STTT	LYM/LYG-ViraStripe STTT	C6-ViraStripe STTT	LYT-C6	LYM/LYG-C6[c]
Lyme disease (124)					
Early Lyme disease with EM					
Acute (40)	19 (48)	17 (43)	17 (43)	20 (50)	22 (55)
Convalescent (38)	24 (63)	23 (61)	24 (63)	30 (79)	29 (76)
Lyme neuroborreliosis or Lyme carditis					
Lyme neuroborreliosis (10)	8 (80)	9 (90)	9 (90)	9 (90)	10 (100)
Lyme carditis (7)	7 (100)	7 (100)	6 (86)	6 (86)	6 (86)
Late Lyme disease, Lyme arthritis (29)	28 (97)	28 (97)	28 (97)	29 (100)	29 (100)
All Lyme disease	86 (69)	84 (68)	84 (68)	94 (76)	96 (77)
Negative controls (347)					
Other diseases (144)					
Fibromyalgia (31)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Severe periodontitis (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Rheumatoid arthritis (21)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Syphilis (20)	0 (0)	0 (0)	0 (0)	2 (10)	1 (5)
Multiple sclerosis (22)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)
Infectious mononucleosis (30)	8 (27)	5 (17)	2 (7)	2 (7)	1 (3)
All other diseases	8 (6)	5 (3)	2 (1)	5 (3)	2 (1)
Healthy controls (203)					
Endemic[d] (101)	3 (3)	5 (5)	1 (1)	1 (1)	1 (1)
Nonendemic[d] (102)	1 (1)	1 (1)	0 (0)	0 (0)	1 (1)
All healthy controls	4 (2)	6 (3)	1 (0)	1 (0)	2 (1)
All negative controls	12 (3)	11 (3)	3 (1)	6 (2)	4 (1)

[a]ViraStripe immunoblots were read using a densitometer.
[b]A ≤30-day duration-of-illness cutoff was applied for IgM Western immunoblotting.
[c]The same results were obtained whether or not the ≤30-day duration-of-illness cutoff was applied to the LYM EIA.
[d]Endemic and nonendemic are as defined in the footnotes to Table 1.

Similar comparisons were performed between the two bioMérieux EIA strategies and the C6 EIA (Table 1; see also Table S1 in the supplemental material). When all samples were compared, there was a significant difference ($P < 0.01$) between the LYM/LYG EIAs (with duration of illness considered) and the C6 EIA due to the number of healthy controls that were called positive by the LYM/LYG EIAs (11% increase). When the LYT EIA was compared to the C6 EIA, there was only a significant ($P < 0.01$) increase (22%) in positive assay results for other diseases when using the LYT EIA. This also resulted in a significant difference ($P < 0.01$) between these two assays when all samples were tested but not when samples from Lyme disease patients or healthy controls were tested.

The difference between the proportion of samples called positive by the combined LYM/LYG EIAs and that by the LYT EIA was calculated to be −3.4% (95% confidence interval [CI] of −7.6% to 0.8%) with a P value of 0.12, indicating that the numbers of samples called positive are similar between the two EIA approaches regardless of whether the samples were true positives or controls (see Table S1 in the supplemental material). The percent agreement between LYM/LYG and LYT for all samples was 80% (95% CI of 77% to 83%) (Table 2), and the percentages of disagreement for each possible result (i.e., LYM/LYG was positive and LYT was negative or LYM/LYG was negative and LYT was positive) were similar at 8% and 10%, respectively. The percent agreement for Lyme disease patient samples ranged from 70% to 100%, with the lowest percentage resulting when samples from early Lyme disease patients with EM were tested (Table 2).

Two-tiered testing. STTT and MTTT results are summarized in Table 3. Two-tiered testing was performed using the combined LYT, the dissociated LYM/LYG, and the C6 EIAs as first-tier tests followed by ViraStripe immunoblot assays (IgM and/or IgG) as second-tier tests. Overall, the sensitivities were similar (68 to 69%) for all combinations.

The overall specificity was slightly lower when using the combined LYT EIA (97% specificity) or the dissociated LYM/LYG assays (97% specificity) than with the C6 EIA (99% specificity). These differences were primarily due to higher cross-reactivity when samples from patients with infectious mononucleosis and healthy controls from areas where Lyme disease is endemic were tested using the LYT and LYM/LYG EIAs.

Pairwise comparisons showed little differences between the various two-tiered strategies where an EIA was followed by immunoblotting (LYT-ViraStripe versus LYM/LYG-ViraStripe, LYT-ViraStripe versus C6-ViraStripe, and C6-ViraStripe versus LYM/LYG-ViraStripe) (see Table S1 in the supplemental material). Significant differences were observed between the LYT-ViraStripe and C6-ViraStripe when all samples ($P = 0.02$) and samples from patients with other diseases ($P = 0.04$) were tested. Specifically, the LYT-ViraStripe approach resulted in more false positives (5% more) when samples from patients with other diseases were tested than when C6-ViraStripe was used (Table 3). There were no significant differences when using the C6-ViraStripe and the LYM/LYG-ViraStripe approach.

Two-EIA MTTT algorithms. The LYT EIA and LYM/LYG EIAs were used as first-tier tests in a two-EIA algorithm that used the C6 EIA as the second-tier test (14, 15). The overall sensitivities for these two combinations were similar, with a 76% sensitivity for the LYT-C6 algorithm and a 77% sensitivity for the LYM/LYG-C6 algorithm, and these were significantly higher ($P < 0.05$) than the sensitivities obtained when using immunoblotting as the second-tier test (Table 3). The overall specificity for both algorithms was 98 to 99%, and this was similar to the specificities obtained when immunoblot assays were used as the second-tier test (specificities of 97 to 99%). The LYT-C6 algorithm was less specific when samples from patients with other diseases were tested (97% specificity) than the LYM/LYG-C6 algorithm (99% specificity). The higher sensitivity obtained using the two-EIA approach was primarily due to an increase in positivity for samples from patients with acute and convalescent Lyme disease (Table 3).

Immunoblot reduction using the dissociated LYM/LYG first-tier EIA. Figure 1 summarizes the number of immunoblot assays that were needed for two-tiered testing when the LYT, LYM/LYG, or C6 EIAs were used as first-tier tests. Figure 1A depicts the workflow for the combined LYT EIA. With this assay, a total of 318 immunoblots were needed due to the 159 positive or equivocal LYT EIAs (the recommended ≤30-day cutoff for IgM immunoblot testing was not considered). When duration of illness was considered for IgM immunoblotting, a total of 247 immunoblots were needed. When the dissociated LYM/LYG EIAs were tested, a total of 233 (27% reduction) or 189 (23% reduction) immunoblots were required when duration of illness was not considered and when it was considered for LYM EIA and IgM immunoblot testing, respectively (Fig. 1B). When C6 was used as a first-tier test (Fig. 1C), the total number of immunoblots that were needed was 224 when duration of illness was not considered for IgM immunoblot testing. This strategy required a 4% decrease (duration of illness was not considered for running the LYM EIA and IgM immunoblot) or 6% decrease (duration of illness was considered for running the LYM EIA and IgM immunoblot) in the number of immunoblots that were needed compared to the number needed when using the LYT/LYM EIAs.

DISCUSSION

Based on the College of American Pathologists (CAP) survey, the combined LYT Vidas EIA has been the most commonly used first-tier EIA for the diagnosis of Lyme disease from 2011 to 2016. bioMérieux proposes several advantages to their dissociated EIAs (LYM and LYG) for their Vidas platform compared to their combined LYT EIA. These include a decreased number of total immunoblots that are needed for second-tier testing, a reduction in costs associated with immunoblots, a potential to deduce information on disease progression (based on IgM and/or IgG positivity), a higher sensitivity and specificity, and a reduction in the level of specialized training that is required to properly perform an EIA compared to immunoblotting. Immunoblot reduction occurs because only the corresponding immunoblot assay (IgM or IgG) for a positive or equivocal LYM and/or

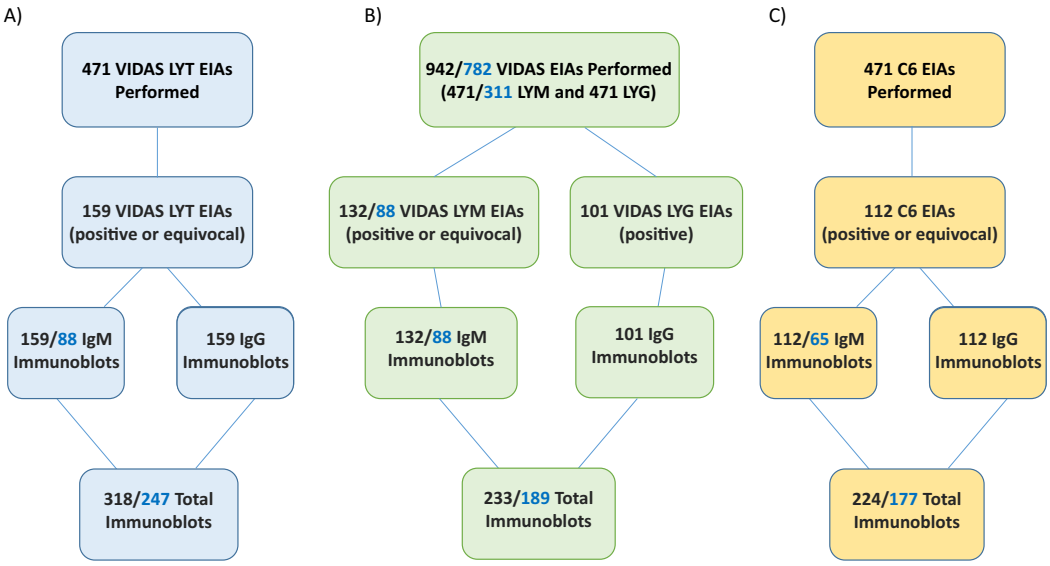

FIG 1 Two-tiered testing using the combined Vidas LYT EIA, the dissociated Vidas LYM and LYG EIAs, and the C6 EIA as first-tier assays. (A) When the combined LYT EIA is used as a first-tier test, all samples that test positive or equivocal are reflexed to both IgM and IgG Western immunoblotting, for a total of 471 first-tier Vidas EIAs and 318 immunoblots needed to perform STTT when the recommended ≤30-day cutoff is not considered for IgM immunoblot testing and 247 immunoblots when duration of illness is considered for IgM immunoblot testing. (B) When the dissociated LYM and LYG assays are used as first-tier tests, the samples that are positive or equivocal by LYM are reflexed to IgM immunoblotting, and the samples that are positive by LYG are reflexed to IgG immunoblotting. The total number of EIAs needed in the first tier is 942 (when duration of illness is not considered for LYM and IgM Western immunoblotting) or 782 (when duration of illness is considered for LYM and IgM Western immunoblotting). The total number of immunoblots needed is 233 or 189 when duration of illness is or is not considered for LYM and IgM Western immunoblotting, respectively. (C) When the C6 EIA is used as a first-tier test, all samples that test positive or equivocal are reflexed to both IgM and IgG Western immunoblotting. A total of 471 first-tier EIAs are used and a total of 224 and 177 immunoblots are used when duration of illness is not and is considered for IgM Western immunoblotting, respectively, to perform STTT. For all two-tiered approaches shown, the numbers in blue take into account the duration of illness of the patients, and only patients having ≤30 days of illness are tested by the LYM EIA and IgM Western immunoblotting.

positive LYG EIA is recommended. This differs from the recommended STTT, where both IgM and IgG immunoblot assays are performed simultaneously following a first-tier positive/equivocal result for patients with ≤30 days of illness.

In this study, we compared the performance of the LYT EIA to that of the LYM and LYG EIAs using the same sample set. It should be noted that the assays were not performed side by side; however, the samples of the LSR were collected and stored with the intent of being used for comparative analysis that may or may not occur at the same time. Additionally, all appropriate assay controls and instrument calibrations were performed according to the manufacturer's recommendations. Our analyses confirmed that for the sample set tested the number of immunoblots needed was less for the dissociated LYM/LYG EIAs than for the LYT EIA, especially when duration of illness was considered for running the LYM EIA. A reduction by 23 to 27% was observed that depended on whether or not the ≤30-day duration-of-illness cutoff was applied for the LYM EIA and IgM Western immunoblot testing. Although immunoblot assays are reduced in the second-tier test, two EIAs need to be performed in the first tier and the total number of tests (first and second tier) required is dependent on the rate of positive first-tier results. Specifically, a total of 942 or 782 EIAs (depending on whether or not a duration-of-illness cutoff was used for LYM EIA testing) were needed for the LYM/LYG approach compared to 471 EIAs when two-tiered testing was done using the LYT or C6 approach. A reduction in the number of immunoblots needed for two-tiered testing was not observed when the LYM/LYG EIA approach was compared to the C6 EIA (Fig. 1).

The information obtained from the use of the LYM and/or LYG EIAs should be correlated with the duration of illness and the patient's history of Lyme disease. It should be noted that there is no recommendation by bioMérieux to not run the

LYM EIA if the patient has a >30-day duration of illness. For patients who have not experienced a previous Lyme disease infection, an initial IgM response within the first weeks of infection followed by an IgG response is expected (16). Therefore, separate IgM and IgG testing should corroborate the patient's illness history.

The overall sensitivities and specificities of the LYT and the LYM/LYG EIAs were similar, although specificity differences between them were observed when certain groups of negative-control subjects were evaluated. Specifically, disagreement was demonstrated when testing healthy control subjects or patients with other diseases. When healthy controls were tested (particularly those from areas where Lyme disease is endemic), the LYM/LYG testing strategy was significantly less specific than the LYT EIA. Of the healthy control samples that had a positive LYM/LYG interpretation, 61% of them were due to an equivocal LYM EIA result. A previous study analyzing the same sample set of healthy controls from areas where Lyme disease is endemic and areas where it is not, all of whom had no known history of Lyme disease, showed that 23% and 31%, respectively, had positive OspC reactivity in an IgM Western immunoblot assay (14). To better understand if OspC may be the cause of reactivity in the LYM assay, a direct comparison between the positive LYM samples and the positive IgM OspC samples by Western immunoblotting was performed; 57% of the LYM-positive healthy control samples (from areas where Lyme disease is endemic and where it is nonendemic) were found to be reactive to OspC in IgM Western immunoblot assays. Given that the LYM EIA uses OspC and DbpA as the test antigens, we postulate that IgM cross-reactivity to OspC may be a cause of false positivity in the LYM EIA. The etiology of this cross-reactivity is unknown at this time and may be due to other underlying conditions present in this group of healthy individuals or perhaps a background level of reactivity due to a previous unknown Lyme disease infection or an infection with a cross-reacting infectious agent. Further studies are under way to assess if the trends observed in this current study will be observed with other healthy control sample sets.

For sample sets from patients with histories of disease other than Lyme disease, the number of LYT EIA false positives was reduced by more than half when samples were tested by the LYM/LYG EIAs. Differences between the two first-tier tests and the increased specificity of the LYM/LYG assay were also linked to certain disease groups, including syphilis, multiple sclerosis, and infectious mononucleosis. For three additional control patient groups (rheumatoid arthritis, severe periodontitis, and fibromyalgia), equivalent or slightly diminished specificity in the LYM/LYG assays compared to the LYT assay was observed. When LYT or LYM/LYG assays were followed by second-tier Western immunoblotting (STTT) or the C6 EIA (MTTT), additional increases in specificity were observed for all combinations tested. These findings further support the value of recommended two-tier testing with available commercial assays. When considering all samples tested by these STTT or MTTT approaches, no significant performance differences were linked to use of one or the other bioMérieux first-tier test. Further, all MTTT combinations outperformed those of STTT for sensitivity. These findings further corroborate that an MTTT approach is a simplified and objective alternative to STTT (14, 15).

When using the LYM/LYG EIAs as a first-tier test followed by the C6 EIA as a second-tier-test, it should be noted that the LYG EIA uses VlsE as one of three test antigens. VlsE is an immunogenic variable surface protein of *B. burgdorferi* that contains two invariable domains at each terminus and a central domain that is composed of six variable and six invariable regions (IRs) (17, 18). C6 is the sixth invariable region (IR6) of the central domain of VlsE (3). The degree to which testing for antibody to these two antigens sequentially leads to independent results is not fully understood. Patients who react to VlsE often also have positive reactivity to C6 (19). However, the IR6 epitopes are largely hidden from the surface of VlsE, and it is predicted that only 13.7% of the IR6 theoretical surface area is solvent exposed (18, 19). Breakdown of the VlsE molecule during infection likely occurs and may expose the C6 domain to the host immune response. The reactive antigens that result in a positive test when a WCS-based EIA is used are undefined and could well be represented as test antigens in the second-tier immunoblot test. In the case of the LYM and LYG EIAs, OspC is one of the test antigens

that is also included on immunoblot assays (IgM and IgG) as an evaluated test antigen. While independence between first- and second-tier tests has not been a requirement for Lyme disease serologic testing, it is important to consider and better understand these parameters in the context of test improvement. Additionally, it should be noted that a high degree of sequence divergence has been reported for VlsE among Lyme disease-causing *Borrelia* species (20). These differences could potentially lead to a false-negative result when using the LYG EIA.

Overall, the dissociated LYM/LYG EIAs performed, with minor exceptions, equivalently to the LYT in test-to-test comparisons or as first-tier assays in STTT or MTTT. An advantage to users who already have the Vidas instrument in their laboratories is that these first-tier EIA approaches use the same automated platform. To the extent that they may reduce the number of Western immunoblots required, this approach will remove technical time and complexity associated with immunoblotting.

MATERIALS AND METHODS

Patient samples. The sera used in this study are from the CDC LSR and were previously described (4). Briefly, the LSR contains serum samples that were stored upon receipt at −80°C in 100-μl aliquots that are from Lyme disease patients and various non-Lyme disease control groups. The purpose of this repository is to provide serum samples to requestors whose work involves the development and testing of novel diagnostic assays for Lyme disease. The Lyme disease patient sera of the LSR are from individuals who had early Lyme disease with a characteristic erythema migrans (EM) rash (acute- and convalescent-phase serum samples; $n = 78$), Lyme neuroborreliosis ($n = 10$), Lyme carditis ($n = 7$), or Lyme arthritis ($n = 29$) at the time of collection. The control sera ($n = 347$) were collected from patients with fibromyalgia ($n = 31$), infectious mononucleosis ($n = 30$), multiple sclerosis ($n = 22$), rheumatoid arthritis ($n = 21$), severe periodontitis ($n = 20$), or syphilis ($n = 20$) and from healthy donors from regions in which Lyme disease is endemic ($n = 101$) or not endemic ($n = 102$). Institutional Review Board approval was granted by the CDC for the testing of these samples.

Serologic tests. Prior to serologic testing, all samples were randomized and coded. Samples were tested by the Vidas Lyme IgM II (LYM) and Vidas Lyme IgG II (LYG) (bioMérieux, Inc., Durham, NC) dissociated assays. For head-to-head performance comparison of the assays, an EIA equivocal result was treated as a positive result. For final two-tier sensitivity and specificity performance, only the corresponding IgM and/or IgG immunoblot assay was considered following a positive or equivocal LYM or positive LYG test.

Previously collected and published data on study samples for the Vidas Lyme IgG and IgM (LYT) assay (bioMérieux, Inc.) (4), the C6 *B. burgdorferi* (Lyme) EIA (Immunetics, Boston, MA), and the ViraStripe IgM and IgG Western immunoblot assays (Viramed Biotech AG, Germany) were used for comparative purposes against the LYM/LYG test results (4, 14). Two-tiered test interpretation was performed according to the recommended guidelines and as previously reported (1, 4, 14).

A duration-of-illness cutoff of >30 days was used when performing the LYM EIA and IgM Western immunoblotting for patients with Lyme disease and other diseases when stated. Duration of illness was established by the clinician through clinical examination of each patient at the time of study enrollment and through the use of a detailed clinical history questionnaire administered to each patient (4).

All serological testing was conducted according to the manufacturer's instructions in the same laboratory by individuals trained to properly perform the assays used. Additionally, the required calibrations and proper test controls were used to ensure accurate instrumentation and run performance.

Statistics. Kappa statistics and their confidence intervals were computed for all serum groups combined to estimate agreement among test results using the LYT and LYM/LYG EIAs as previously described (14). McNemar's test for difference in proportions was used to test for and estimate the difference in percent correct for the various testing strategies evaluated, and this was also performed as previously described (14).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.02407-16.

SUPPLEMENTAL FILE 1, XLSX file, 0.1 MB.

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