Evaluation of Two-Test Serodiagnostic Method for Early Lyme Disease in Clinical Practice

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The Centers for Disease Control and Prevention (CDC) recommend a two-test approach for the serodiagnosis of Lyme disease (LD), with EIA testing followed by Western immunoblotting (WB) of EIA-equivocal and -positive specimens. This approach was compared with a simplified two-test approach (WB of EIA equivocals only) and WB alone for early LD. Case-patients with erythema migrans (EM) rash ⩾5 cm were recruited from three primary-care practices in LD-endemic areas to provide acute- (S1) and convalescent-phase serum specimens (S2). The simplified approach had the highest sensitivity when either S1 or S2 samples were tested, nearly doubling when S2 were tested, while decreasing slightly for the other two approaches. Accordingly, the simplified approach had the lowest negative likelihood ratio for either S1 or S2. For early LD with EM, the simplified approach performed well and was less costly than the other testing approaches since less WB is required.

Lyme disease (LD), caused by the tickborne spirochete *Borrelia burgdorferi* sensu lato, is the most common vectorborne illness in the United States [1]. For surveillance purposes, the Centers for Disease Control and Prevention (CDC) define a confirmed LD case as (1) a case with a physician-diagnosed erythema migrans (EM) rash of ⩾5 cm or (2) a case with at least one late manifestation (musculoskeletal, neurologic, or cardiovascular disease) that is laboratory-confirmed [2]. Serologic testing is often used in clinical practices in the diagnosis of LD, although it has been complicated by inappropriate and excessive use, with resulting difficulty in interpretation of test results [3–5].

The need for standardized methods for serologic diagnosis of LD became apparent after studies that demonstrated poor intra- and interlaboratory agreement of LD serologic results [6–9]. Consequently, the CDC assembled a panel of serum specimens from well-characterized LD patients and controls from areas endemic and nonendemic for LD for evaluation and standardization of EIA and Western immunoblotting (WB) procedures for detecting antibodies to *B. burgdorferi.* Initial studies demonstrated the value of WB in resolving the interpretation of equivocal EIA results that would otherwise have been scored as negative, thereby increasing sensitivity without a loss of specificity [9]. This gave impetus to further evaluate the adjunctive role of WB in LD serodiagnosis. Subsequent studies included testing of patients with a low pretest likelihood of LD, including those with such potentially cross-reactive conditions as syphilis or tickborne relapsing fever [10, 11]. Such studies demonstrated the ability of WB to discriminate many false-positive EIA reactions, and led to the adoption of the CDC-recommended two-test approach (CDC-recommended approach) at the Second National Conference on the Serologic Diagnosis of Lyme Disease [12, 13]. In this approach, specimens are first tested by using a sensitive EIA or indirect IFA. Equivocal or positive serum specimens are then tested with the more specific IgM and IgG WB. The rationale for WB of EIA-positive serum specimens was to maintain a high specificity, especially for testing serum specimens from patients with nonspecific clinical findings and for patients from areas not known to be LD-endemic [14].

The current study reexamines the CDC-recommended approach in primary-care practices in LD-endemic areas, where it is likely that most LD tests are requested. This study design allowed the evaluation of test performance among case-patients with a high pretest likelihood of early LD. Accordingly, the objectives of this study were to (1) compare the test perform-

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Written informed consent was obtained from all case-patients and controls or their guardians. The guidelines of the Centers for Disease Control and Prevention and the Connecticut Children's Medical Center were followed in the investigation with regard to human subjects review.

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ance of the CDC-recommended approach to two other testing approaches: WB alone and a simplified two-test approach (simplified approach) in which only EIA equivocals are followed by WB, and (2) evaluate test performance in primary-care practices in areas highly endemic for LD.

Methods

Selection of case-patients. Three primary-care family practices in LD-endemic areas (Connecticut, Massachusetts, and Rhode Island) were selected as study sites. During the June–September 1996 transmission season, on-site health-care providers experienced in the diagnosis of LD were instructed to recruit case-patients who met the following study case definition for early LD: physician-diagnosed EM rash ⩾5 cm.

During the initial office visit, case-patients were requested to provide an acute-phase serum specimen (S1) and to complete a standardized questionnaire to collect information on personal risk factors, environmental exposures, illness history, and history of vaccination for LD. Health care providers were requested to complete a standardized questionnaire to record the examination findings and the medical history obtained at the initial office visit. All case-patients were treated with antibiotics at the time of the initial office visit and were requested to return in 4 weeks for reevaluation of illness and collection of a convalescent-phase serum specimen (S2). Case-patients were excluded if they did not meet the study case definition or if they received LD vaccine as part of a vaccine clinical trial. Case-patients of any age were eligible for inclusion in the study. Blood specimens were collected in serum separator tubes and processed at the University of Connecticut Health Center. Frozen serum specimens were sent to the CDC for testing.

Selection of controls. Control serum specimens were obtained during the 1995 LD transmission season from voluntary participants in an annual Block Island, Rhode Island, serosurvey. Participants in the serosurvey were asked to complete a standardized questionnaire to collect information on personal risk factors, environmental exposures, illness history, and history of vaccination for LD. The following persons were excluded as controls: those reporting a previous diagnosis of LD; a history of rash, fever, chills, myalgia, fatigue, or joint pain and/or swelling in the past year; or a history of vaccination for LD as part of a vaccine clinical trial. Persons of any age were eligible to be enrolled as controls, as long as they had resided on Block Island for at least 1 month during the transmission season. Control blood specimens were collected, processed, and tested in the same manner as case-patient specimens.

Specimen testing. Blinded serum specimens were submitted to the CDC in a coded, unlinked fashion by the University of Connecticut Health Center. Serum specimens were tested at the CDC by a polyvalent (IgM/IgG) EIA (Vidas; BioMérieux Vitek, Hazelwood, MO) and separate IgM and IgG WB (Marblot; MarDx Diagnostics, Carlsbad, CA) according to the manufacturers' instructions. Low-passage *B. burgdorferi,* strain B31, was used as the antigen source for both assays. Serum specimens with at least 2 of 3 IgM diagnostic bands or 5 of 10 IgG diagnostic bands by WB were considered positive, in accordance with the CDC-recommended criteria for WB interpretation [13]. The following antigens were also evaluated for IgM and IgG immunoblots, although they are not included in the above criteria: 60 kDa, 62 kDa, 37 kDa, 34 kDa (OspB), and 31 kDa (OspA). In accordance with the CDC-recommended WB criteria, only IgG WB results were considered in the evaluation of control serum specimens and case-patient serum specimens collected>30 days after illness onset [13]. The identity of specific antigens in immunoblots was facilitated by the use of reference monoclonal antibodies [14].

Statistical analysis. Data were entered into Microsoft Access (version 2.0) and imported into Epi Info (version 6.04b) for descriptive epidemiologic and χ^2 analyses. The intertest agreement between the CDC-recommended approach, simplified approach, and WB alone was performed using PEPI (version 2.07A) to calculate the κ statistic, a proportional measure of agreement which corrects for chance [15].

Results

Of 91 case-patients recruited for the study, 74 were determined to be eligible for enrollment in the study. Ten case-patients were excluded because they did not meet the study case definition, 5 because of insufficient clinical information to determine if the study case definition was met, and 2 for having received LD vaccine as part of a vaccine clinical trial. Of the eligible study participants, 41 (55%) were male and 33 (45%) female, and the median age was 41 years (range, 3–83). S1 were collected from 66 case-patients a median of 4 days after illness onset (range, 0–19), and S2 were collected from 55 case-patients a median of 36 days after illness onset (range, 21–161). Symptoms and potential exposures reported by the case-patients are summarized in table 1.

Control serum specimens. Serum specimens were obtained from 80 serosurvey participants who had no reported history of LD; 38 of these were determined to be eligible for enrollment in the study. Twenty controls were excluded for having received LD vaccine as part of a vaccine clinical trial and 22 because of a history of rash, fever, chills, myalgia, fatigue, or swollen

Table 1. Signs, symptoms, and exposures reported by case-patients (n = 74).

Outcome or exposure	Number (%)
Exposure to potential tick habitat in 30 days before illness onset	70 (94.6)
Bitten by tick in 30 days before illness onset	23 (31.1)
Fatigue	42 (56.8)
Myalgia	32 (43.2)
Headache	29 (39.2)
Chills	26 (35.1)
Joint pain	26 (35.1)
Measured temperature >37°C	23 (31.1)
Multiple erythema migrans rashes	10 (13.5)
Lymphadenopathy	10 (13.5)
Joint swelling	8 (10.8)
Radiculoneuritis	1 (1.4)
Keratitis	1 (1.4)

NOTE. No cardiac involvement, lymphocytic meningitis, cranial neuritis, or encephalomyelitis was diagnosed in any case-patients.

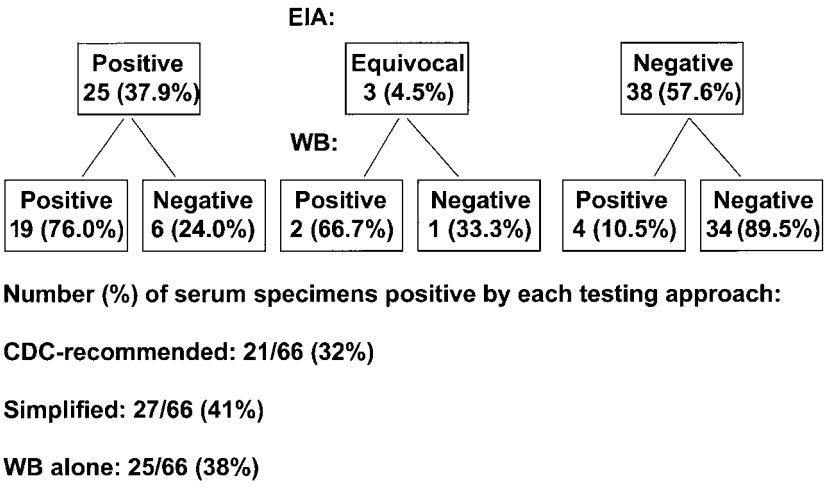


Figure 1. Results of EIA and Western immunoblotting (WB) of 66 acute-phase (S1) serum specimens

or painful joints in the previous year. Of the 38 eligible controls, 17 (45%) were male and 21 (55%) female, and the median age was 58 years (range, 11–82). By serology, 37 controls (97%) were EIA-negative; 1 of these (3%) was also IgG WB–positive. The 1 remaining specimen was EIA-equivocal and IgG WB–negative.

Case-patient serum specimens. The results of serologic testing of S1 and S2 are presented in figures 1 and 2, respectively. Test performance characteristics, when S1 and S2 were tested using the CDC-recommended approach, the simplified approach, and WB alone, are presented in table 2.

Sensitivity and intertest agreement by day of collection. The sensitivity of the three testing approaches was compared by the interval (days) from illness onset to serum specimen collection (figure 3). The sensitivity was 39% for both the simplified approach and WB alone for serum specimens collected 0–14 days after onset but was highest for the simplified approach for serum specimens collected either 15–29 or ⩾30 days after illness onset (table 3). The sensitivity was highest for serum specimens collected 15–29 days after illness onset, regardless of the testing approach used. The simplified approach was significantly more sensitive than the CDC-recommended approach for serum specimens collected ⩾30 days after onset ($P < .05$). Otherwise, the sensitivity of either the simplified approach or WB alone did not significantly differ from the CDC-recommended approach. Only the sensitivity of the simplified approach demonstrated a significant χ^2 test for trend over time ($P < .01$).

κ statistics calculated for the three testing approaches demonstrated high intertest agreement for serum specimens collected 0–14 days after illness onset (table 3). There was a decline

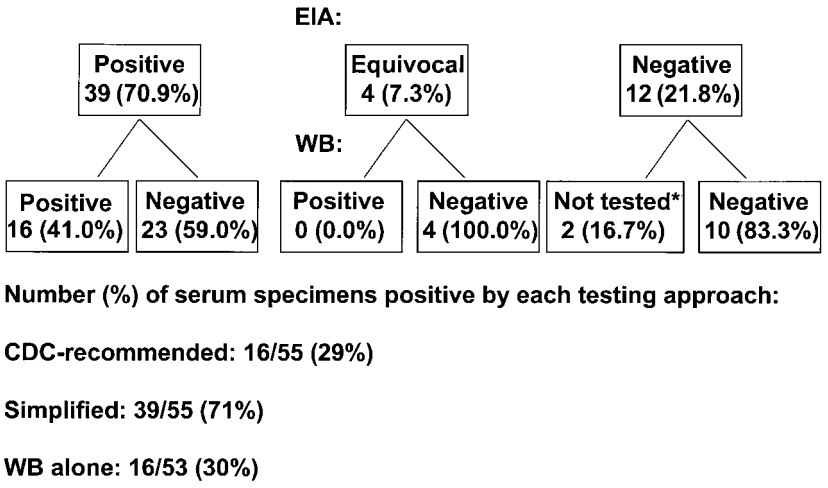


Figure 2. Results of EIA and Western immunoblotting (WB) of 55 convalescent-phase specimens (S2). * Insufficient quantity.

Table 2. Test performance characteristics of the CDC-recommended approach, the simplified approach, and Western immunoblotting of all serum specimens.

	CDC approach	Simplified approach	Western immunoblotting alone
Specificity	100%	100%	97%
S1 sensitivity	32%	41%	38%
S2 sensitivity	29%	71%	30%
S1 LR^+	∞	∞	14.6
S2 LR^+	∞	∞	11.5
S1 LR^-	0.68	0.59	0.64
S2 LR^-	0.71	0.29	0.72

NOTE. S1, acute-phase specimens; S2, convalescent-phase specimens; LR, likelihood ratio; LR^+, sensitivity/1− specificity; LR^-, 1−sensitivity/specificity.

in intertest agreement for subsequent time intervals for the CDC-recommended approach versus the simplified approach and the simplified approach versus WB alone. Four serum specimens collected 0–14 days after illness onset were positive by WB alone but negative by the CDC-recommended approach, resulting in a slightly lower κ for the CDC-recommended approach versus WB alone for this time interval, compared with subsequent time intervals.

WB reactivity. The frequency of WB reactivity to specific antigens is shown in table 4. IgG antibody to the 41-kDa antigen (flagellin) was detected in at least 66% of both case-patient and control serum specimens. However, IgM reactivity to this antigen was significantly greater among case-patients than controls for both S1 and S2 ($P < .01$). Most case-patients demonstrated reactivity to two or more antigens within 30 days of illness onset by both the IgG and IgM WB (figure 4). However, only 12 case-patients (16%) ever developed an IgG response sufficient to meet the CDC-recommended WB criteria.

Discussion

The primary objective of this study was to compare the test performance of the CDC-recommended approach with two other approaches. Overall, for case-patients with suspected early LD with EM, the simplified approach had the best performance characteristics compared with the CDC-recommended approach and the use of WB alone. Consistent with these findings, a two-test approach, in which only EIA equivocals are tested by WB, was used as the basis of the recently published American College of Physicians (ACP) guidelines for laboratory evaluation in the diagnosis of LD [16].

A secondary objective of this study was to evaluate testing approaches among early LD case-patients who are representative of those seen in primary-care practices. Previous evaluations of the sensitivity and specificity of the CDC-recommended approach included serum specimens from highly selected, and in many cases culture-confirmed, LD case-patients [9, 10, 17]. Although the authenticity of these serum specimens is well documented, they may reflect only a subset of serum specimens collected in primary-care practices. The population used to evaluate the three testing approaches in the current study is representative of case-patients meeting the CDC surveillance case definition for early LD, with physician-diagnosed

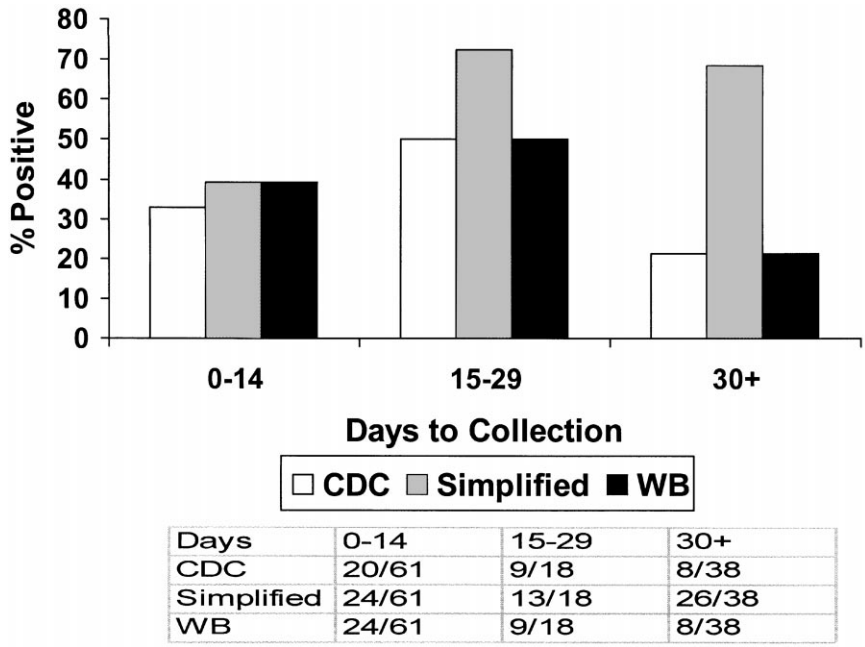


Figure 3. Sensitivity of CDC-recommended and simplified approaches and Western immunoblotting alone by interval from illness onset to serum specimen collection date (days).

Table 3. Sensitivity and intertest agreement of the CDC-recommended approach, the simplified approach, and Western immunoblotting (WB) alone by the interval from illness onset to serum specimen collection date.

Days	CDC approach	Simplified approach	WB alone	CDC vs. simplified κ (95% CI)	CDC vs. WB alone κ (95 % CI)	Simplified vs. WB alone κ (95% CI)
0–14	20/61 (33)	24/61 (39)	24/61 (39)	0.86 (0.73–0.99)	0.86 (0.73–0.99)	0.73 (0.55–0.90)
15–29	9/18 (50)	13/18 (72)	9/18 (50)	0.56 (0.17–0.95)	1.0 (1.0–1.0)	0.56 (0.17–0.95)
⩾30	8/38 (21)	26/38 (68)	8/37 (22)	0.22 (−0.20–0.64)	1.0 (1.0–1.0)	0.21 (−0.22–0.64)

NOTE. Data in columns 2–4 are no. positive/total tested (sensitivity in %). CI, confidence interval.

EM rash ⩾5 cm, who present to primary-care practices in areas in which LD is highly endemic. This clinical case definition was used as the reference standard in the current study, against which performance characteristics of the three testing approaches were compared.

The simplified approach was evaluated as a means of reducing the number of WB performed in circumstances in which EIA-positives are considered adequate supportive evidence to guide diagnosis and management. Reducing the number of WB tests, while ensuring a high level of specificity, would reduce the cost and labor associated with the serologic diagnosis of LD. A recent survey in Maryland found that the mean commercial laboratory charge for WB for LD was $106, compared with $52 for EIA [18]. Accordingly, costs associated with the CDC-recommended approach, simplified approach, and WB alone in the current study would have been $13,818, $7,034, and $12,614, respectively.

The use of WB alone was evaluated to examine the necessity of the first testing step. Some patients and health care providers may perceive that WB is a "superior" test and request it alone in support of a diagnosis of suspected LD. In addition, some health care providers may request WB alone in order to limit the number of tests ordered per patient, for reasons such as restrictions placed by insurance companies or health maintenance programs, or simply the desire for a more straightforward testing approach. The sensitivity of WB alone did not differ significantly from the CDC-recommended approach, whereas other measures of test performance, such as likelihood ratios, positive (LR^+) and negative (LR^-), were inferior or comparable to those achieved using either the CDC-recommended or simplified approach.

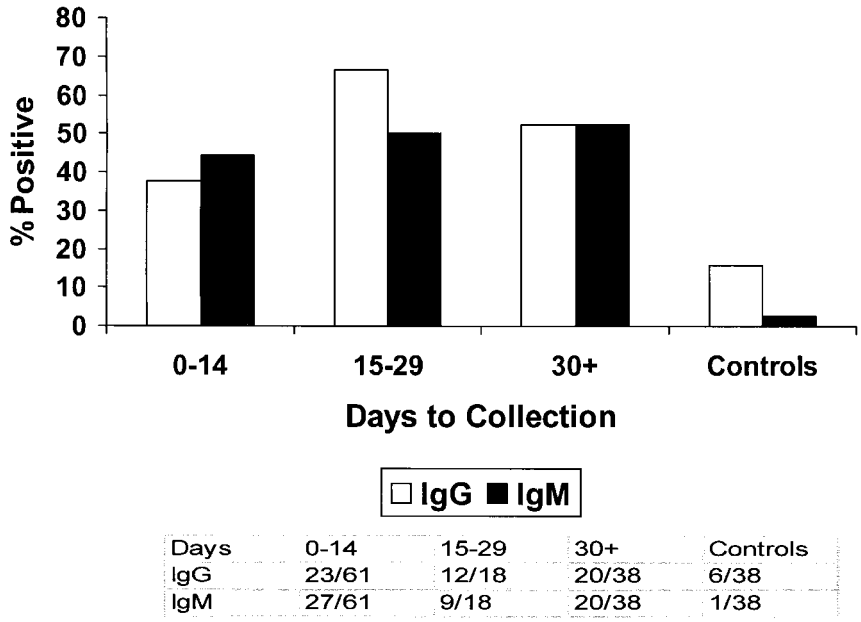
Table 4. Frequency of Western immunoblot bands among acute- (S1) and convalescent-phase (S2) serum specimens from case-patients and serum specimens from controls.

	Case-patients					
	S1[a] (n = 66)		S2[b] (n = 53)		Controls (n = 38)	
Band	IgM	IgG	IgM	IgG	IgM	IgG
93	12 (18)	5 (8)	8 (15)	5 (9)	2 (5)	2 (5)
66	11 (17)	6 (9)	11 (21)	8 (15)	1 (3)	3 (8)
62	6 (9)	4 (6)	3 (6)	7 (13)	1 (3)	1 (3)
60	13 (20)	9 (14)	10 (19)	14 (26)	0	3 (8)
58	10 (15)	6 (9)	6 (11)	11 (21)	0	1 (3)
45	3 (5)	15 (23)	1 (2)	15 (28)	0	2 (5)
41	30 (45)	45 (68)	32 (60)	41 (77)	3 (8)	25 (66)
39	11 (17)	5 (8)	8 (15)	11 (21)	3 (8)	2 (5)
37	10 (15)	3 (5)	1 (2)	3 (6)	0	0
34	3 (5)	1 (2)	1 (2)	1 (2)	0	0
31	3 (5)	2 (3)	2 (4)	1 (2)	1 (3)	0
30	0	2 (3)	0	0	0	0
28	0	2 (3)	1 (2)	4 (8)	0	1 (3)
23	29 (44)	16 (24)	32 (60)	23 (43)	4 (11)	0
18	1 (2)	7 (11)	5 (9)	10 (19)	0	2 (5)

NOTE. Data are no. positive for band (%).
[a] S1 collected 0–19 days after illness onset.
[b] S2 collected 21–161 days after illness onset.

The American College of Physicians guidelines recommend serologic testing only when the pretest probability of LD is 0.20–0.80, as evidenced by clinical findings and the incidence of LD in the population represented by the patient [16]. There is currently no widely used, standardized method for quantifying the pretest likelihood of infection. The pretest likelihood of LD for case-patients enrolled in the current study is estimated to be very high because of the presence of physician-diagnosed EM and the case-patients' residence in areas in which LD is highly endemic [19]. However, the percentage of seropositive case-patients was relatively low in each of the testing approaches evaluated. Seronegativity among case-patients when S1 were tested may be due to sampling prior to the development of antibodies against *B. burgdorferi* or to misdiagnosis of other skin lesions as EM; either could result in differential misclassification of case-patients [20, 21]. Some case-patients may have had EM-like lesions from a tickborne agent other than *B. burgdorferi* [22]. Seronegativity among case-patients when S2 were tested may be due to a diminished immune response in case-patients who receive early antibiotic treatment [23]. Given the relatively low level of seroreactivity observed in the current study, antibiotic treatment of persons with EM on the basis of exposure history and clinical presentation, rather than on results of serologic testing, seems prudent. The ideal serologic testing approach remains to be determined for LD case-patients from LD-endemic areas who do not have physician-diagnosed EM, but have objective clinical findings that are otherwise compatible with LD.

Controls were selected to represent residents in a highly LD-endemic area who do not have a history of LD. We felt that the use of controls from an endemic area would best determine test performance in an area where the test would most appropriately be applied. Eligible controls reported no symptoms compatible with LD in the previous year and had no history of a LD diagnosis. This healthy control group may underrepresent the range of test reactivity displayed by patients with



Days	0-14	15-29	30+	Controls
IgG	23/61	12/18	20/38	6/38
IgM	27/61	9/18	20/38	1/38

Figure 4. Proportion of serum specimens from case-patients, by interval from illness onset to specimen collection date (days), and controls, with 2 IgG or IgM bands.

other illnesses whose clinical findings overlap with those of LD, resulting in an overestimation of test specificity. In previous studies, the CDC-recommended approach has demonstrated 100% specificity in large numbers of nonendemic controls and 90% specificity for persons with illnesses that may cause cross-reactivity [10, 24]. The possibility remains that some controls previously experienced subclinical infection, or that persons volunteering for the LD serosurvey availed themselves of the free serologic testing because they were at an increased risk for LD. In spite of the potential for misclassification bias, only 1 control had a positive IgG WB according to the CDC-recommended WB criteria, whereas 6 demonstrated reactivity to two or more antigens in the IgG WB.

LRs were calculated, using both sensitivity and specificity, to provide useful and realistic indicators of serologic test performance for the three testing approaches evaluated in the current study. LRs allow the diagnostician to determine how much a given test result will raise (LR^+) or lower (LR^-) the pre- to posttest likelihood of disease [25]. The simplified approach had the highest sensitivity and specificity when either S1 or S2 were tested, resulting in a lower negative LR compared with the other two approaches. Accordingly, a negative result obtained by using the simplified approach is more likely to be a true negative than one obtained by using the CDC-recommended approach or WB alone. The LR^+ was ideal (infinity) for both the CDC-recommended and simplified approaches when either S1 or S2 were tested, since the specificity was 100% for both approaches (table 1). The low specificity and sensitivity of WB alone contributed to a lower LR^+ for this approach. The lower specificity of WB alone resulted from 1 control serum specimen that was EIA-negative but IgG WB–positive and did not differ significantly from the specificity of the other two approaches ($P > .5$). The sample size of the current control group ($n = 38$) may be too small to detect differences in specificity between the testing approaches.

The sensitivity was lower for S2 than S1 when either the CDC-recommended approach or WB alone was used, going from 32% to 29% and 38% to 30%, respectively. This is the result of S2 collected >30 days after illness onset that were IgM WB–positive and IgG WB–negative. In accordance with the CDC's recommendations, IgM WB results were not considered in the evaluation of serum specimens collected >30 days after illness onset, as a heightened and more specific IgG response is expected in this time frame [13]. Thus, positive IgG WB results were required to determine seropositivity for specimens collected >30 days after illness onset, whereas both IgG and IgM WB results were evaluated for serum specimens collected before this time. Thirteen case-patients had serum specimens collected >30 days after illness onset that were IgM WB–positive, but IgG WB–negative, according to the CDC-recommended WB criteria. Twelve of these case-patients had available S1, of which 6 (50%) demonstrated seroconversion on both EIA and IgM WB, 2 (17%) on EIA only, and 1 (8%) on IgM WB only. Three of the 12 had no evidence of seroconversion. For case-patients with both S1 and S2 available, the sensitivity may be increased for serum specimens collected >30 days after illness onset by including seroconversion as a criterion when evaluating IgM WB results, relaxing the stringency of the IgG

WB criteria, or both. The interpretation of paired serum specimens is currently not addressed by the CDC recommendations for test performance and interpretation [13].

Intertest agreement was compared for the three testing approaches using the κ statistic, a proportional measure of agreement that corrects for chance [15]. If observed agreement is greater than or equal to that expected by chance, $\kappa \geq 0$, and if there is complete agreement, $\kappa = 1$. The intertest agreement was low for the simplified approach versus the CDC-recommended approach and the simplified approach versus WB alone for serum specimens collected >30 days after illness onset. This is likely due to the low sensitivity of the IgG WB for serum specimens collected >30 days after illness onset in this study population.

Results of the current study and others suggest that increased serologic test sensitivity may be achieved by modification of some of the CDC-recommended criteria for WB interpretation. For instance, one study of 100 case-patients with early LD (EM rash $\geq$5 cm) found that 65 had WB reactivity, although only 43 (66%) of these were interpreted as positive by the criteria of Dressler et al. [26]. An investigation of 46 culture-confirmed patients by Aguero-Rosenfeld et al. [27] found that 89% of the patients developed measurable IgG responses during the 1-year follow-up period, even though only 22% ever met the CDC-recommended IgG WB criteria. Similarly, in the current study, only 12 (16%) of 74 case-patients ever manifested an IgG response sufficient to meet the CDC-recommended WB criteria, compared with 41 (58%) who demonstrated $\geq$2 diagnostic IgG bands. A separate study, also focused on serodiagnosis of early LD, proposed that the presence of any 2 IgM WB reactive bands in patients with EM is a more sensitive criterion than the CDC-recommended WB criteria [28]. Implementation of these interpretive criteria in the current study resulted in the detection of 6 additional case-patients within 30 days of illness onset by IgM WB.

Inclusion of the 31- and 34-kDa antigens in the WB criteria has also been recommended as a means of increasing the sensitivity of this test [29]. However, other studies indicate that these antigens are poorly expressed during early infection, and that their inclusion in WB interpretive criteria does not significantly improve test sensitivity [11, 30, 31]. In the current study, inclusion of these two antigens to the CDC-recommended WB criteria would have resulted in the detection of only 1 additional case-patient by IgM WB. Although previous studies have proposed blotting criteria with novel sets of antigens, with or without the use of band intensity cutoffs, it was not the objective of this study to perform such comparative evaluations [32–34]. Furthermore, such comparisons are hampered by the lack of widely available markers to certain antigens and standardized densitometry.

Implementation of the two-test approach to serologic testing for LD may include the use of assays that are unlike those used in the current study. For instance, some laboratories may repeat an equivocal EIA result to "verify" the result. However, this does not obviate performing a WB as the second step on such specimens. Similarly, a WB should follow equivalent results obtained by a monovalent EIA.

Improving the performance of serologic testing for LD remains a challenge. Among the testing approaches compared in the current study, the simplified two-test approach demonstrated superior test performance and cost savings in early LD case-patients with EM in LD-endemic areas. However, both first-step tests and WB require further standardization. It is also likely that the results of EIAs based on whole cell–antigen preparations will be confounded in recipients of OspA LD vaccine [35, 36]. Further evaluations of the simplified approach are needed among LD vaccine recipients; patients from LD-endemic areas with clinical findings compatible with LD but without EM; and patients with neurologic, musculoskeletal, or cardiac manifestations of LD, to further define the role of this testing approach in the serologic diagnosis of LD.

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