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Lyme Disease Testing by Large Commercial Laboratories in the United States

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Abstract

Background—Laboratory testing is helpful when evaluating patients with suspected Lyme disease (LD). A two-tiered antibody testing approach is recommended, but single-tier and non-validated tests are also used. We conducted a survey of large commercial laboratories in the United States to assess laboratory practices. We used these data to estimate the cost of testing and number of infections among patients from whom specimens were submitted.

Methods—Large commercial laboratories were asked to report the type and volume of testing conducted nationwide in 2008, as well as the percent of positive tests for four LD endemic states.

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DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

The total direct cost of testing was calculated for each test type. These data and test-specific performance parameters available in published literature were used to estimate the number of infections among source patients.

Results—Seven participating laboratories performed ~3.4 million LD tests on ~2.4 million specimens nationwide at an estimated cost of $492 million. Two-tiered testing accounted for at least 62% of assays performed; alternative testing accounted for less than 3% of assays. The estimated frequency of infection among patients from whom specimens were submitted ranged from 10% to 18.5%. Applied to the total numbers of specimens, this yielded an estimated 240,000 to 444,000 infected source patients in 2008.

Discussion—LD testing is common and costly, with most testing in accordance with diagnostic recommendations. These results highlight the importance of considering clinical and exposure history when interpreting laboratory results for diagnostic and surveillance purposes.

Keywords

Lyme disease; Infection; United States; Diagnostic Testing; Cost

INTRODUCTION

Lyme disease (LD) is caused by *Borrelia burgdorferi*, a bacterium transmitted through the bite of infected *Ixodes* species ticks. Nearly 30,000 confirmed cases were reported to the Centers for Disease Control and Prevention (CDC) in 2008 [1], ranking LD among the ten most commonly reported nationally notifiable diseases in the United States (U.S.). LD is a geographically focal illness, occurring predominantly in the Northeastern and North Central states.

LD diagnosis is based on clinical manifestations and the potential for exposure to infected ticks [2]. For the majority (70%–80%) of cases, the disease begins with a characteristic erythema migrans (EM) rash and accompanying flu-like symptoms [3]. Left untreated, *B. burgdorferi* can disseminate over days to weeks, and develop into multiple EM rashes, acute neuroborreliosis (e.g., meningitis, facial palsy, or radiculopathy), or Lyme carditis. After months, untreated LD may manifest as intermittent attacks of oligoarticular arthritis [4, 5]. After months to years, untreated LD may develop into late neuroborreliosis (e.g., Lyme encephalopathy, radiculoneuropathy or parasthesias) [6–11].

Serologic testing can be helpful when evaluating patients with suspected LD. CDC recommends a two-tiered approach to LD serologic testing [12]. The first tier consists of an immunoassay (ELISA/EIA) using whole-cell, recombinant, or synthetic peptide antigens or, rarely, an immunofluorescense assay (IFA). If the results of the first test are positive or indeterminate, supplementary Western blots for IgG or IgM anti-*Borrelia burgdorferi* antibodies are performed to increase testing specificity. As with other serologic tests, the sensitivity and specificity of this two-tiered approach varies by stage of disease. Two-tiered testing is relatively insensitive (<40%) during early illness, characterized by EM rash. It is reasonably sensitive (>87%) and specific (99%) when used for diagnostic testing of disseminated LD [13]. For this reason, CDC recommends this two-tiered approach primarily

for patients having signs and symptoms of disseminated disease. Although not generally recommended or cleared by the U.S. Food and Drug Administration (FDA), alternative tests (e.g., PCR, urine antigen test) are used by some providers [14].

In this paper, we present the results of a survey regarding LD testing performed by large commercial laboratories in the U.S. These data were originally collected as part of a larger survey of tickborne diseases (N Connally, Hinckley A, Meek J, *et al.*, manuscript in preparation). Collaborators included investigators participating in the TickNET program, a network of public health partners created in 2007 to foster collaboration on surveillance, research, education, and prevention for tickborne diseases. Primary outputs of the current study were: (1) total number and type of LD tests performed nationwide by large commercial laboratories, and (2) percent positive for tests submitted from four states where LD is endemic. We used these data to establish a baseline for laboratory testing practices, compare reported practices to published recommendations, estimate the cost of LD testing in the U.S., and provide a national estimate of the number of infections among source patients from whom samples were submitted.

METHODS

Representatives from seven large commercial laboratories (ARUP, Clinical Laboratory Partners, Focus Diagnostics, Laboratory Corporation of America (LabCorp), Mayo Clinic Laboratories, Quest Diagnostics, and Specialty Laboratories) were asked to participate. These laboratories accounted for greater than 76% of LD tests reported to health departments in the four endemic states (Connecticut, Maryland, Minnesota and New York), in 2008. In addition, we attempted to include laboratories known to provide alternative methods of LD testing. Representatives at participating laboratories were asked to complete a written survey regarding the number of tests performed by their laboratory in 2008 for 14 different serologic assays or assay combinations (e.g., ELISA/EIA with reflex to Western blot), seven PCR tests distinguished by specimen type (blood, skin, CSF/synovial fluid, urine, semen, breast milk, and other), and four culture tests distinguished by specimen type (skin, synovial fluid, skin/synovial fluid, or other). In addition, respondents were asked to report the number of direct visualization, urine antigen, CSF antibody and any other diagnostic tests performed for LD. Respondents were asked to report the percent positive by diagnostic assay for residents of four endemic states (CT, MD, MN, and NY). The number of tests and percent positive by test type were compiled across all laboratories. This research was considered exempt from Human Subjects Review by Centers for Disease Control and Prevention and Yale University ethics committees.

To estimate the total direct cost of LD testing, median charges by commercial laboratories for each of the following test types were obtained from Wormser et al. [15]: whole-cell ELISA ($127), C6 ELISA ($180), IgM and IgG Western blot tests ($264 combined). These costs were applied to the number of reported tests. For this analysis, we assumed the IFA test to equal the cost of whole-cell ELISA, and the individual Western blot tests to cost exactly half ($132) of the total combined test. We calculated the cost of two-tiered testing as the cost of the first tier plus the cost of the second tier when first tier testing was positive.

The frequency of positive results as reported by laboratories reflects a combination of true positive, false positive and false negative test results. Therefore, to estimate the true frequency of infection among all specimens submitted to the seven participating laboratories, it was necessary to correct the reported rate of positive tests (observed percent positive) for the sensitivity and specificity of the assays used (see online supplement for methods). The result, the percent of true infections, was multiplied by the total number of specimens tested by participating laboratories to estimate the total number of infections among source patients nationwide.

RESULTS

All seven large commercial laboratories agreed to participate; none of the laboratories known to perform alternative testing agreed to participate. Responding laboratories performed a total of 3,351,732 LD tests on 2,432,396 specimens in 2008 (Table 1). Sixty-two percent of tests were conducted using a two-tiered approach and 38% were conducted as standalone tests. As individual tests, PCR, CSF antibody, standalone C6 peptide ELISA, IFA, culture, and urine antigen tests accounted for ≤ 1% of assays performed. For Western blot tests alone (without preceding ELISA/EIA), 48% were IgM Western blots, 49% were IgG Western blots, and three percent were IgM/IgG combination Western blot tests.

Laboratory testing in the four endemic states accounted for 1,053,445 (or 31%) of tests conducted nationwide by participating laboratories. For comparison, these four states accounted for 36% of all LD cases reported to CDC during 2007–2009 [1, 16, 17]. The majority (68%) of tests were two-tiered ELISA/EIA with Western blot reflex (Table 2). As with the national data, ≤1% of each of the following diagnostic LD tests was conducted: PCR, CSF antibody test, standalone ELISA (whole-cell and C6 peptide), and culture. There were no urine antigen tests conducted for residents of these states. Five laboratories (responsible for >48% of all tests conducted by participating laboratories) reported complete data on percent positive for all test types for the four states. Aggregate results from these laboratories are presented in Table 2. For the two-tiered tests, the percent positive for tests from the four states was 5.8% when using the ELISA/EIA as a first tier. For the Western blot standalone tests, 10.5% were positive for IgM antibodies and 6.5% for IgG. Standalone ELISA/EIA tests were positive on 11.4% of sera. The percent positive was lowest (≤3.1%) for PCR, CSF antibody, and culture.

Given an overall frequency of positive first-tier assays of 11.89% (Table 3), the estimated total direct cost for two-tiered tests was approximately $336 million. Additionally, expenditures for standalone Western blot, and ELISA/IFA/C6 tests totaled $117, and $39 million, respectively. Taken together, these figures amount to a total of $492 million.

Presented in Table 3 are the frequency of positive two-tiered and EIA/ELISA tests reported by five national laboratories for specimens from CT, MD, MN, and NY. Also included are the parameters used to estimate the percent of true infections [18–23]. The overall estimated percent of true infections among patients for whom samples were tested was 12% (see online supplement). Sensitivity analysis indicates that this overall value is robust, remaining relatively stable regardless of the proportion of specimens derived from patients with early

versus later stages of infection. When the data were evaluated for individual states by laboratory, the estimated percent of true infections varied from 10% in MD to 18.5% in MN. Multiplying these percentages by the total number of specimens tested yielded an estimate of 288,000 infected source patients in the U.S., with a range of 240,000 to 444,000.

DISCUSSION

In this survey, we found that approximately 3.4 million LD tests were conducted by participating laboratories in 2008, at an estimated cost for laboratory services of $492 million. Most LD testing was in accordance with current recommendations; at least 62% of tests conducted nationwide utilized the two-tiered procedure recommended by the U.S. Public Health Service Agencies and the Infectious Diseases Society of America [2]. For samples tested by Western blot alone, it is possible that some were first evaluated by EIA/ELISA at a smaller (e.g., hospital-based) laboratory before being sent to a participating laboratory. Therefore, the true percentage of samples tested using a two-tiered approach may be higher than 62%. These results may not be generalizable to laboratories that did not participate in the survey. In particular, the frequency of testing by alternative methods is expected to be higher at laboratories that did not participate in the survey.

A previous analysis using marketing data from 1995 estimated that over $100 million dollars were spent annually on LD tests [24–26]. In a more geographically limited study, Strickland et al. reported that nearly 30,000 LD tests were conducted for Maryland residents in 1995, at a cost of over $2 million [27]. We estimated a total direct laboratory cost of $492 million. This value reflects the amount charged by commercial laboratories that is ultimately paid by insurance companies, Medicare/Medicaid, the patient, and/or the ordering medical center (e.g. hospitals, clinics). It does not include additional handling charges that may be incurred by patients, discounts offered by laboratories, charges for PCR or other less common tests, or tests performed by non-participating laboratories. Recently, Branda et al. proposed using two EIAs as an alternative to the standard two-tiered approach as a means of preserving the sensitivity and specificity of testing, while reducing costs [28]. Based on our findings, this approach would reduce the national cost estimate by approximately $57 million per year.

Overall, participating laboratories tested 2.4 million specimens for LD. When multiplied by the estimated percent of true infections (12%), this yields 288,000 infected source patients in the U.S., approximately ten times higher than the number of cases reported to CDC in 2008. Underreporting is a common feature of routine surveillance, and the values here are consistent with what has been previously reported for LD [29–32]. It should be noted that our estimate of the percent of true infections was relatively insensitive to assumptions regarding the frequency of early infection among source patients (see online supplement).

Our estimate of infected source patients is subject to several limitations. First, the observed percent positive is based on samples from four states. The remaining samples are assumed to have come mostly from patients in other endemic states who have a similar risk of infection. This assumption is supported by the observation that the four states account for 31% of all samples and 36% of LD cases reported nationwide, confirming that diagnostic samples are generated in proportion to where the disease occurs. Second, we used percent positive values

for tests conducted by only five large commercial laboratories in four endemic states (representing ~15% of nationwide data). These were the only laboratories that provided complete responses to all questions. Results from these laboratories may not be representative of results from all other large commercial laboratories, and the percent positive values found for these states may not be representative. However, together, these laboratories conducted a substantial proportion (>45%) of all the tests performed by the participating laboratories. Lastly, to calculate the percent of true infections we computed an average value for sensitivity and specificity for each test using data presented in the published literature. This method does not account for the variability that might exist between laboratories or test kits.

Two study limitations may have led to overestimating the number of infected source patients. While we assumed that one specimen was submitted for every patient, it is likely that multiple specimens may have been submitted for at least some patients. For example, if 85% of patients had a single specimen, 10% had two specimens, and 5% had three specimens tested, then the overall estimate would be reduced by 17%. Also, the estimate is potentially influenced by individuals who were seropositive as a result of previous infection but did not have active infection at the time of testing. At least one study indicates that some successfully treated individuals can maintain a seropositive status for up to 10 years [33].

There are also several factors to suggest that 288,000 is an underestimate of the total number of infections that occurred in the U.S. in 2008. First and foremost, it does not address infection in individuals for whom no testing is sought (i.e. persons diagnosed clinically based upon presence of an EM rash) or for whom no testing data was available. In addition, this estimate is based upon numbers of specimens submitted for LD testing at only those participating laboratories (the source population). Our analysis did not consider testing done at smaller laboratories, clinics, hospitals, etc. Data from a related survey indicates that these smaller facilities account for at least another 14% of tests run for residents of the four endemic states (unpublished data, CDC). Serologic testing for the diagnosis of LD has been complicated by inappropriate and excessive use and may be a substantial misuse of healthcare resources [34, 35]. Even when serologic testing is ordered in endemic areas, it may be unwarranted clinically. The low percent positive values reported in this study for tests conducted in four endemic states support this claim. A study by Fix *et al*. found that the majority of patients who presented with a tick bite had serologic tests ordered for the detection of antibodies to *B. burgdorferi*, though none of the patients ever developed LD symptoms [36]. Serologic tests conducted for LD at the time of tick bite are not useful because the patient has not yet developed a detectable antibody response to infection. Given the large volume of testing nationally, small differences in test specificity would be expected to have a large impact on the number of false positive results generated, possibly promoting misdiagnosis. False-positives may occur more often for self-referred patients, and for those presenting with non-specific symptoms from non-endemic areas where the pretest likelihood of disease is low [37, 38]. Such results can lead to unnecessary antibiotic treatment, which in turn may be associated with adverse events [24, 39].

This survey of laboratories has provided a baseline for laboratory testing practices and the cost of LD testing in the U.S., and has provided a national estimate of the number of

infected source patients. Given the large number of tests for LD and potential for false results, it is important to consider clinical and exposure history in conjunction with laboratory results for diagnosis and classification of LD for surveillance purposes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ARTICLE SUMMARY

Large commercial laboratories in the United States were surveyed to determine Lyme disease testing frequency, practices, and results. Approximately 3.4 million tests were conducted in 2008; 62% in accordance with recommendations. We estimate that 288,000 infections occurred among 2.4 million patients from whom samples were submitted.

Table 1

Lyme disease tests conducted in the U.S. at large commercial laboratories, by test type, 2008[1].

Test Type	Tests Conducted	%
Two-Tiered Approaches		
Whole-Cell ELISA/EIA[2]	2,026,117	60
C6 Peptide ELISA[2]	71,257	2
Standalone Tests		
Western blot	887,616	26
Whole-cell ELISA/EIA	298,058	9
C6 Peptide ELISA	3,790	<1
IFA – IgG	2,031	<1
IFA - IgM	1,101	<1
PCR	40,761	1
CSF Antibody	20,908	1
Culture	74	<1
Urine Antigen	19	<1
Total	3,351,732	

[1] Data aggregated from seven U.S. commercial laboratories.

[2] Denotes reflex to supplementary Western blot.

Table 2

Lyme disease tests at large commercial laboratories by type and percent positive, in four states, 2008[1]

	No. of Tests	% of Tests	% Positive[2]
	701,006	68	5.8
Two-Tiered Whole Cell ELISA/EIA or C6 ELISA[3]			
Standalone Tests			
IgG Western blot	155,800	15	6.5
IgM Western blot	155,584	15	10.5
IgM/IgG Combination	82	<1	-
Whole Cell ELISA/EIA	3,616	<1	11.4
C6 peptide ELISA	1,655	<1	-
PCR[4]	7,637	<1	3.1
CSF Antibody[5]	3,055	<1	-
Culture	7	<1	0
Total	1,053,445		

[1] Data aggregated from seven U.S. commercial laboratories. Includes specimen testing data from providers in Connecticut, Maryland, Minnesota, and New York.

[2] Phase 1 %positive results are presented from five laboratories. CSF antibody not performed by these laboratories.

[3] Positive results for C6 (standalone) not reported.

[4] PCR testing of blood, cerebrospinal fluid, and/or synovial fluid.

[5] No urine antigen tests were reported for these four states.

Table 3

Lyme disease testing volume data, sensitivity and specificity values, and observed and predicted percent positive by test for large commercial laboratories in four endemic states

Test Type	Number of Tests	Sensitivity-Localized Disease[1]	Sensitivity-Disseminated Disease[1]	Specificity	Observed % Positive[2]	Predicted % Positive[3]	Predicted % Difference
Two-Tiered Whole Cell	297,619	37.0%	87.0%	99.4%	5.79	5.76	0.03
EIA/IFA standalone or first tier[4]	287,595	66.9%	93.3%	96.1%	11.89	11.89	0.00

[1] Se and Sp values derived from listed references [6, 18–23, 25]

[2] Observed percent positive values were derived by combining data from five large commercial laboratories for residents of four endemic states (CT, MD, MN, NY; see online supplement)

[3] Predicted percent positive values were iteratively derived using data on the number of tests performed, Se and Sp of tests, an estimate for proportion of tests run from patients having localized/early disease, and an estimate for the true rate of infection (see online supplement)

[4] Based on EIA/IFA standalone combined with first tier of two-tiered assays