Laboratory Blood-Based Testing for Non-Lyme Disease Tick-Borne Infections at a National Reference Laboratory

A Seven-Year Experience

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Key Words: Babesiosis; Anaplasmosis; Ehrlichiosis; Rocky Mountain spotted fever; Tularemia; Tick-borne relapsing fever; Colorado tick fever

Am J Clin Pathol January 2020;153:139-145

DOI: 10.1093/AJCP/AQZ139

ABSTRACT

Objectives: *We evaluated trends in non-Lyme disease tick-borne disease (NLTBI) testing at a national reference laboratory.*

Methods: *Testing data performed at Quest Diagnostics during 2010 to 2016 were analyzed nationally and at the state level.*

Results: *Testing and positivity for most NLTBIs increased dramatically from 2010 through 2016 based on testing from a large reference laboratory. The number of positive cases, though not as stringent as criteria for public health reporting, generally exceeds that reported by the Centers for Disease Control and Prevention. The frequency of NLTBI in the US is seasonal but testing activity and positive test results are observed throughout all months of the year. Positive results for NLTBI testing mostly originated from a limited number of states, indicating the geographic concentration and distribution of NLTBIs reported in this study.*

Conclusions: *This report provides an important complementary source of data to best understand trends in and spread of NLTBI.*

Tick-borne infections (TBIs) include a variety of bacterial, viral, and parasitic pathogens whose incidence varies across different regions of the United States.[1] While Lyme disease is by far the most common TBI in the United States,[2] there are many non-Lyme TBIs (collectively called NLTBIs), including babesiosis, anaplasmosis, ehrlichiosis, Rocky Mountain spotted fever (RMSF), tularemia, tick-borne relapsing fever (TBRF), and Colorado tick fever (CTF) among others. According to recent data from the Centers for Disease Control and Prevention (CDC), the number of reported cases of many NLTBIs has increased significantly from 2004 to 2016.[3] For example, reported cases of anaplasmosis and ehrlichiosis combined increased by 6.6-fold from 875 to 5,750 in 13 years. Likewise, in a previous study on TBI testing performed at the Massachusetts General Hospital (MGH), a large urban academic medical center in Boston, we demonstrated an increase in testing volume during 2006 to 2016 and an increase in the number of positive tests for all TBI illnesses endemic to this region.[4] The absolute increase in NLTBIs may attribute to better reporting, more widely available and improved clinical laboratory testing, greater awareness of NLTBI, or a combination of many factors. However, the published data have suggested there is significant underreporting of Lyme disease and Q fever to CDC.[5,6] As TBIs mostly use the comparable reporting system as Lyme disease, potentially, underreporting also occurs for TBIs other than Lyme disease and Q fever. In a study on Lyme disease testing at Quest Diagnostics, a

national reference laboratory, we observed an increasing trend in Lyme disease testing and positivity in recent years, including in areas not historically associated with high rates of the disease.[7] In tandem with the Lyme disease study, in this study we examine the same trends in NLTBI testing performed at Quest Diagnostics over a 7-year period (2010-2016) and evaluate potential underreporting of NLTBIs by comparing our results with the numbers of cases reported to the CDC during the same period. About 3,000 public health departments gather data on these diseases. Through the National Notification of Diseases Surveillance System the CDC receives these data. Reporting responsibility varies for different health departments.

Materials and Methods

This study included NLTBI disease data of all patients aged 0 to 89 years from the Quest Diagnostics database from 2010 through 2016. No testing was performed at the MGH. All data were derived from the Quest Diagnostics national database.

Testing for NLTBI at Quest Diagnostics includes polymerase chain reaction (PCR) and serological tests as listed below. All PCR assays are laboratory-developed tests (LDT) at Quest Diagnostics and reported as not detected or detected. Serological tests are also LDTs except for RMSF, and their analytical performance characteristics are determined by Quest Diagnostics. All serological tests use immunofluorescence assays except for tularemia, which uses direct agglutination. For each serological test, a reference range is established during test development, which is the screening dilution for an assay. In this analysis, if a reported numeric titer is above the reference range, the result is classified as positive. The following LDTs have not been cleared or approved by the US Food and Drug Administration (FDA) but their assays have been validated pursuant to the Clinical Laboratory Improvement Amendments regulations.

1. Babesiosis PCR and serology (LDT, slides from Diasorin Molecular). Babesiosis PCR is a highly specific (no known cross-reactivity) and sensitive (100% analytical sensitivity) method to detect the presence of *Babesia microti* DNA. DNA sequences from the closely related canine pathogen *B gibsoni* are not detected by this assay. Reference range: <1:64 for *B microti* antibodies IgG[8] and <1:20 for *B microti* antibodies IgM.[9]
2. Anaplasmosis PCR and serology (LDT, slides from Diasorin Molecular). Anaplasmosis DNA PCR test (no known cross-reactivity, 100% detection rate for analytical sensitivity) is to detect the agent responsible for human granulocytic anaplasmosis. This assay does not recognize sequences for the human ehrlichial pathogen *Ehrlichia chaffeensis*. Reference range: <1:64 for *Anaplasma phagocytophilum* antibodies IgG[10] and <1:20 for *A phagocytophilum* antibodies IgM.[11]
3. Ehrlichiosis PCR and serology (LDT, slides from Diasorin Molecular). Ehrlichiosis PCR is a highly sensitive method to detect the presence of *E chaffeensis* DNA in clinical specimens. This assay cannot differentiate between viable and nonviable organisms. Reference range: <1:64 for *E chaffeensis* antibodies IgG[12] and <1:40 for *E chaffeensis* antibodies IgM (by industry standard and based on the distribution of reported results in the Quest Diagnostics database).
4. RMSF serology (FDA cleared kit from Diasorin Molecular, product code IF0112/ IF0114 for *Rickettsia* spotted fever IgG/IgM positive control; Focus Diagnostics). Quest Diagnostics performs a two-stage approach to the serological assessment for RMSF: first a screening IgG-IgM test and, if positive, this is followed by a confirmatory titer. Reference range for screening *Rickettsia* (RMSF) antibodies IgG/IgM is not detected; for confirmatory titer <1:64 for IgG/IgM (by Package Insert for FDA-cleared tests).
5. Tularemia serology (LDT, antigen from Becton Dickinson). Reference range: <1:20 for *Francisella tularensis* antibodies.
6. TBRF serology (LDT, slides from Focus Diagnostics). Reference range: <1:64 for *Borrelia hermsii* antibodies IgG and <1:20 for *B hermsii* antibodies IgM.
7. CTF (LDT, slides from Diasorin Molecular). Reference range: <1:16 for CTF antibodies IgG and <1:20 for CTF antibodies IgM.

Quest Diagnostics does not offer testing for Powassan virus. Testing performed on nonblood-based specimens was not included in this study. During the study period, there were no major changes in test methodology that would affect assay performance or to the menu of available tests or test systems used at Quest Diagnostics that may lead to a sudden increase or decrease in test volume. Data were stratified by test year, test month, and state of residence. This study is part of the ongoing Quest Diagnostics Health Trends, an effort deemed exempt by the Western Institutional Review Board (Puyallup, Washington).

Simple linear regression based on ordinary least squares, test year being the only explanatory variable in the model, was used to assess the monotone increasing or decreasing trends in test volume and number of positives over time. Statistical analyses were performed in SAS Studio 3.6 on SAS 9.4 (SAS Institute).

DOI: 10.1093/ajcp/aqz139

Table 1
Babesiosis

	Polymerase Chain Reaction		Serology			
Year	No. Tests	Positives, No. (%)	No. Tests, Paired IgG-IgM	Positives IgG ≥ 1:64, No. (%)	Positives IgM ≥ 1:20, No. (%)	No. Babesiosis Cases Reported to NNDSS[a]
2010	2,710	109 (4.0)	37,604	1,155 (3.1)	1,476 (3.9)	NA
2011	3,770	116 (3.1)	45,910	2,551 (5.6)	1,779 (3.9)	1,128
2012	4,155	114 (2.7)	55,389	2,454 (4.4)	1,287 (2.3)	937
2013	5,078	225 (4.4)	58,532	2,568 (4.4)	1,799 (3.1)	1,796
2014	7,777	283 (3.6)	60,252	2,371 (3.9)	1,588 (2.6)	1,760
2015	9,791	262 (2.7)	62,825	1,671 (2.7)	1,041 (1.7)	2,100
2016	11,579	270 (2.3)	71,971	2,208 (3.1)	1,877 (2.6)	1,910
Total	44,860	1,379 (3.1)	392,483	14,978 (3.8)	10,847 (2.8)	9,631

NA, not available; NNDSS, National Notification of Diseases Surveillance System.
[a]In source reference[3] surveillance data for babesiosis reported during 2011 to 2016.

Table 2
Anaplasmosis

	Polymerase Chain Reaction		Serology			
Year	No. Tests	Positives, No. (%)	No. Tests, Paired IgG-IgM	Positives IgG ≥ 1:64, No. (%)	Positives IgM ≥ 1:20, No. (%)	No. Anaplasmosis Cases Reported to NNDSS[a]
2010	606	50 (8.3)	31,836	2,049 (6.4)	734 (2.3)	1,875
2011	1,220	93 (7.6)	37,758	2,689 (7.1)	658 (1.7)	2,736
2012	1,943	86 (4.4)	40,486	2,883 (7.1)	468 (1.2)	2,597
2013	2,339	111 (4.7)	51,137	4,121 (8.1)	1,204 (2.4)	3,033
2014	3,707	194 (5.2)	55,760	3,239 (5.8)	673 (1.2)	3,013
2015	4,542	240 (5.3)	57,653	2,920 (5.1)	1,006 (1.7)	3,849
2016	4,927	244 (5.0)	66,636	3,540 (5.3)	1,546 (2.3)	4,373
Total	19,284	1,018 (5.3)	341,266	21,441 (6.3)	6,289 (1.8)	21,476

NNDSS, National Notification of Diseases Surveillance System.
[a]In source reference[3] and https://www.cdc.gov/ehrlichiosis/stats/index.html.

Results

Babesiosis

Over the 7-year study period, 44,860 PCR tests and 392,483 paired IgG-IgM serological tests for *Babesia* species were performed, of which 1,379 (3.1%) PCR, 14,978 (3.8%) IgG, and 10,847 (2.8%) IgM tests were reported positive **Table 1**. From 2010 to 2016, the annual volume increased by 4.3-fold ($P < .001$) in PCR tests and 1.9-fold ($P < .001$) in serological tests. Over the same period, the number of positive cases more than doubled ($P < .01$) in PCR and the positivity rate fluctuated between 2.3% and 4.4% in a random pattern. In serology, the number of positive tests varied but did not show a monotone trend, with a positivity rate between 2.7% and 5.6% for IgG and 2.3% and 3.9% for IgM. Six states (New York, Minnesota, Connecticut, Massachusetts, New Jersey, and Rhode Island) accounted for 92.8% and 87.6% of the positive IgG and IgM tests, respectively.

Anaplasmosis

Testing by PCR for anaplasmosis increased 8.1-fold from 606 in 2010 to 4,927 in 2016 ($P < .001$), while the volume of serological tests increased by 2.1-fold ($P < .0001$) from 31,836 in 2010 to 66,636 in 2016 **Table 2**. The number of positive tests increased 4.9-fold ($P < .001$) in PCR but the rate of positivity showed a modest decline over 2010 to 2016 from 8.3% to 5.0% ($P = .08$). In serological tests, there was no monotone increasing trend in the number of positive tests or the positivity rate over time. Overall, 21,441 (6.3%) IgG and 6,289 (1.8%) IgM tests were positive. Six states (New York, Minnesota, Connecticut, Massachusetts, New Jersey, and Wisconsin) accounted for 82.2% of positive IgM and 79.5% of positive IgG tests.

Ehrlichiosis

Overall, 26,830 PCR tests and 489,043 paired IgG-IgM serological tests were performed, with an increase of 2.4-fold ($P = .02$) in PCR tests and 1.7-fold ($P < .01$) in serological tests from 2010 to 2016 **Table 3**. However, the number of positive tests varied from a high of 133 to a low of 48 without an apparent trend in PCR, while it remained unchanged in serological tests. Similarly, the rate of positivity in PCR varied from a low of 1.0% to a high of 3.0%, also without an apparent trend. Overall, 16,549 (3.4%) IgG and 2,099 (0.4%) IgM tests were positive. Seven states (New York, Arkansas, Missouri, New Jersey,

DOI: 10.1093/ajcp/aqz139

Table 3
Ehrlichiosis

Year	Polymerase Chain Reaction: No. Tests	Polymerase Chain Reaction: Positives, No. (%)	Serology: No. Tests, Paired IgG-IgM	Serology: Positives IgG ≥ 1:64, No. (%)	Serology: Positives IgM ≥ 1:40, No. (%)	No. Ehrlichiosis Cases Reported to NNDSS[a]
2010	2,300	48 (2.1)	49,381	2,346 (4.8)	453 (0.9)	740
2011	3,057	86 (2.8)	53,697	1,310 (2.4)	325 (0.6)	850
2012	4,059	109 (2.7)	71,609	2,271 (3.2)	220 (0.3)	1,128
2013	4,361	133 (3.0)	73,171	3,724 (5.1)	333 (0.5)	1,518
2014	3,912	104 (2.7)	75,915	2,494 (3.3)	212 (0.3)	1,475
2015	3,671	63 (1.7)	80,884	2,271 (2.8)	290 (0.4)	1,288
2016	5,470	57 (1.0)	84,386	2,133 (2.5)	266 (0.3)	1,377
Total	26,830	600 (2.2)	489,043	16,549 (3.4)	2,099 (0.4)	8,376

NNDSS, National Notification of Diseases Surveillance System.
[a]In source reference[3] and https://www.cdc.gov/ehrlichiosis/stats/index.html.

Table 4
Rocky Mountain Spotted Fever Two-Stage Serology (Screening Followed by Confirmatory Titer)

Year	IgG: Screening: No. Tests	IgG: Screening: Detected, No. (%)	IgG: Confirmatory Titer: Positives ≥1:64, No. (%)	IgM: Screening: No. Tests	IgM: Screening: Detected, No. (%)	IgM: Confirmatory Titer: Positives ≥1:64, No. (%)	No. Spotted Fever Rickettsiosis Cases Reported to NNDSS[a]
2010	38,576	9,504 (24.6)	9,498 (99.9)	38,575	1,090 (2.8)	1,087 (99.7)	1,985
2011	40,506	11,525 (28.5)	11,523 (100.0)	40,503	1,021 (2.5)	1,019 (99.8)	2,802
2012	51,583	15,575 (30.2)	15,543 (99.8)	51,594	2,465 (4.8)	2,456 (99.6)	4,470
2013	45,443	6,185 (13.6)	6,060 (98.0)	45,447	933 (2.1)	894 (95.8)	3,359
2014	46,586	4,658 (10.0)	4,611 (99.0)	46,587	620 (1.3)	598 (96.5)	3,757
2015	48,355	4,814 (10.0)	4,792 (99.5)	48,307	1,312 (2.7)	1,302 (99.2)	4,198
2016	49,614	4,252 (8.6)	4,244 (99.8)	49,615	339 (0.7)	336 (99.1)	4,269
Total	320,663	56,513 (17.6)	56,271 (99.6)	320,628	7,780 (2.4)	7,692 (98.9)	24,840

NNDSS, National Notification of Diseases Surveillance System.
[a]In source reference[3] spotted fever rickettsiosis (includes *Rickettsia rickettsii*, *R. parkeri*, and *Rickettsia rickettsii* species 364D) reported during 2010 to 2016.

Table 5
Tularemia Serology (Antibody by Direct Agglutination)

Year	No. Tests	Positives ≥1:20, No. (%)	No. Tularemia Cases Reported to NNDSS[a]
2010	7,878	567 (7.2)	124
2011	8,744	218 (2.5)	166
2012	12,062	367 (3.0)	149
2013	11,790	1,102 (9.3)	203
2014	10,484	1,482 (14.1)	180
2015	9,980	2,310 (23.1)	314
2016	10,274	1,803 (17.5)	230
Total	71,212	7,849 (11.0)	1,366

NNDSS, National Notification of Diseases Surveillance System.
[a]In source reference[3] tularemia reported during 2010 to 2016.

Massachusetts, Minnesota, and Connecticut) accounted for 64.8% of positive IgG and 60.9% of positive IgM tests.

Rocky Mountain Spotted Fever

Over 7 years, of 320,663 IgG screening tests performed, 56,513 (17.6%) were reported detected; among those detected, 56,271 (99.6%) were confirmed with an IgG titer equal to or greater than 1:64 **Table 4**. During the same time, of 320,628 IgM screens performed, only 7,780 (2.4%) were detected; among detected IgM screens, 7,692 (98.9%) IgM confirmatory titers were equal to or greater than 1:64 (Table 4). In IgG screening, there was an increase in annual volume but a decrease in annual positive cases, both marginally ($P = .07$), which led to a significant drop in positivity rate from 24.6% in 2010 to 8.6% in 2016 ($P = .01$).

In IgM screening, the same marginal increase was seen in test volume ($P = .07$) but neither positive cases nor positivity rate showed a monotone trend ($P > .1$). Of the

DOI: 10.1093/ajcp/aqz139

Table 6
Tick-Borne Relapsing Fever Serology[a]

Year	No. Tests, Paired IgG-IgM	Positives IgG ≥ 1:64, No. (%)	Positives IgM ≥ 1:20, No. (%)
2010	872	221 (25.3)	5 (0.6)
2011	1,314	314 (23.9)	5 (0.4)
2012	1,908	543 (28.5)	2 (0.1)
2013	2,157	577 (26.8)	7 (0.3)
2014	2,995	1,119 (37.4)	1 (0.0)
2015	2,569	497 (19.3)	3 (0.1)
2016	2,627	303 (11.5)	1 (0.0)
Total	14,442	3,574 (24.7)	24 (0.2)

[a]In source reference[3] tick-borne relapsing fever was not reported.

Table 7
Colorado Tick Fever Serology[a]

Year	No. Tests, Paired IgG-IgM	Positives IgG ≥ 1:16, No. (%)	Positives IgM ≥ 1:20, No. (%)
2010	288	40 (13.9)	5 (1.7)
2011	292	31 (10.6)	5 (1.7)
2012	252	22 (8.7)	7 (2.8)
2013	291	36 (12.4)	7 (2.4)
2014	358	19 (5.3)	6 (1.7)
2015	461	20 (4.3)	8 (1.7)
2016	23	1 (4.3)	— (0.0)
Total	1,965	169 (8.6)	38 (1.9)

[a]In source reference[3] Colorado tick fever was not reported.

IgG-positive results, 55.1% were from six leading states (Oklahoma, Arkansas, Missouri, New York, Tennessee, and Georgia). Similarly, about half of positive IgM were from almost the same set of leading states (Oklahoma, New York, Texas, Tennessee, Arkansas, and Georgia).

Tularemia

Overall, 71,212 serological tests for tularemia were performed from 2010 to 2016, of which 7,849 (11.0%) were reported positive (titers ≥ 1:20), with higher positivity rates in recent years from 2014 to 2016 ($P = .02$) **Table 5**. There was not a monotone temporal trend in test volume but the number of positive tests increased by 3.2-fold from 567 in 2010 to 1,803 in 2016 ($P < .01$). Overall, 71.6% of all positive tests originated from six states (Minnesota, Arkansas, Oklahoma, Missouri, New Jersey, and Massachusetts).

Tick-Borne Relapsing Fever

Table 6 shows 14,442 paired IgG-IgM tests for TBRF were reported over 7 years, of which 3,574 (24.7%) showed a IgG titer equal to or greater than 1:64 and only 24 (0.2%) IgM titer equal to or greater than 1:20. The tests volume appeared to increase by 3.0-fold from 872 to 2,627 over 2010 to 2016 ($P < .01$), but there was not a clear trend in positive cases or positivity rate over time. The greatest number of positive test results were observed from California (37.2% of all positive tests for IgG, 29.2% for IgM) followed by Utah (8.6% for IgG, 12.5% for IgM), New Jersey (6.8 % for IgG, 8.3% for IgM), and Massachusetts (5.9% for IgG, 8.3% for IgM).

Colorado Tick Fever

Positive serological tests for CTF were relatively uncommon, 8.6%% for IgG and 1.9% for IgM on average **Table 7**, and these were concentrated into a limited number of states (Utah 29.6% for IgG and 15.8% for IgM, Minnesota 17.2% for IgG and 34.2% for IgM, and Massachusetts 11.2% for IgG).

Discussion

In this study, tests for seven NLTBIs (babesiosis, anaplasmosis, ehrlichiosis, RMSF, tularemia, TBRF, and CTF) performed at a national reference laboratory over 2010 to 2016 were analyzed in terms of trends of testing volume and positive cases based on PCR and/or serological tests. We observed a surge in testing volume in most NLTBI diseases with an increase of at least twofold over the 7-year study period. The increasing trend in positive cases was seen in babesiosis (PCR only), anaplasmosis (PCR only), and tularemia (serology). The positivity rate over time was variable across all NLTBIs. The observation of positive cases did not support the CDC data[3] or

DOI: 10.1093/ajcp/aqz139

the data collected at MGH.[4] For example, data from CDC indicate an increase in cases of anaplasmosis/ehrichiosis from 2,615 to 5,750 during 2010 to 2016 whereas data from MGH showed the number of cases in the Massachusetts region increased significantly in 2006 to 2012 but then showed a modest decease in 2013 to 2016.

As in the study by Hinckley et al,[5] our data are based on the results of laboratory testing rather than reported cases of NLTBI. We cannot differentiate false-positive test results from true infections nor distinguish between true-negative and false-negative results. A negative PCR result indicates the absence of the target DNA at detectable levels in the sample tested and does not exclude the diagnosis of disease. A positive PCR result should be considered in conjunction with clinical presentation and additional established clinical tests. Also, the test volumes do not distinguish isolated tests on individual patients from patients who may have received multiple tests such as acute and convalescent sera. Ideally, laboratory criterion for serological confirmation of diagnosis requires a fourfold rise in antibody titer in paired acute and convalescent specimens. In this study, we used a single titer instead of paired samples due to data limitations. Such data based on testing volumes and rates of positivity serve only as a proxy to assess overall trends in NLTBI rather than an accurate measure of the actual number of cases. Another limitation is that the market share of Quest Diagnostics is not uniform across the United States. These limitations not withstanding, we believe our data provide useful insights on the frequency and trends in NLTBI across the United States. Specific observations are as follows.

The numbers of positive results in NLTBIs observed in this study are consistently higher than the cases reported to the CDC,[3] suggesting underreporting of NLTBIs. There were 9,631 babesiosis cases reported to CDC from 2011 to 2016 compared with 13,823 positive IgG test results (titer ≥ 1:64) during the same period. Again, 29,852 cases of anaplasmosis and ehrlichiosis combined were reported to CDC in 2010 to 2016, while 37,990 positive test results were identified using IgG test only for both diseases over the same time. In RMSF, 56,271 IgG positive results from screening test were confirmed with an IgG titer equal to or greater than 1:64 in Table 4 compared with 24,840 cases reported to CDC, which indicates the CDC data represent only 44% of positive results identified in this study. For tularemia, the 7,849 positive results from serology test at Quest Diagnostics was 5.7 times higher than those reported to CDC (1,366) during 2010 to 2016. No CDC data were reported for TBRF or CTF. Given that Quest Diagnostics does not perform all NLTBI testing nationally and that the CDC data contain both confirmed and probable cases using clinical and laboratory criteria,[3] it would be reasonable to assume that the extent of underreporting is greater than that described here even after adjusting for the limitations of laboratory data explained above. For TBIs, reporting is generally required by physicians who have direct access to patient history and are aware of clinical symptoms. Like other reportable conditions, TBIs have different reporting requirements in different states. Variation can also exist due to diseases to be reported, time frames for reporting, agencies receiving reports, persons required to report, and report formats.

Positive results for NLTBI serological testing mostly originated from a limited number of states, indicating the geographic concentration and distribution of NLTBIs reported in this study largely were consistent with those reported by CDC.[13] Babesiosis (>88%) and anaplasmosis (>80%) were mostly from the populous northeastern states (New York, Connecticut, Massachusetts, New Jersey, and Rhode Island) and two Upper Midwestern states (Minnesota and Wisconsin). Ehrlichiosis (>61%) was also found in the populous Northeastern states (New York, New Jersey, Massachusetts, and Connecticut) as well as in Arkansas, Missouri, and Minnesota. CDC stated over 60% of RMSF cases in 2016 were reported from five states (North Carolina, Oklahoma, Arkansas, Tennessee, and Missouri)[13] and our data showed 46% of IgG and 37% IgM positive results for RMSF were from these five states from 2010 to 2016. Tularemia has been reported from all states except for Hawaii according to CDC,[13] while in this study three leading states (Minnesota, Arkansas, and Oklahoma) accounted for 55% of all positive serological test results with a titer equal to or greater than 1:20. Among 14 Western states in which TBRF most commonly occurs, as identified by CDC, California and Utah were the two foremost states in this study, comprising 46% of IgG and 42% of IgM positive results. CTF was primarily detected from Utah and Minnesota in our data, jointly representing about half of all positive results.

The frequency of NLTBI in the United States is seasonal but testing activity and positive test results are observed throughout all months of the year (data not shown). More positive serological tests occurred in summer than in winter, from a high of 2,858 of 2,401 in July to a low of 497 of 309 in February, using babesiosis IgG/IgM testing in this study as an example. This peak occurrence in summer months was also seen in other NLTBIs in this study, although a significant number of positive tests occurred in winter months as well. This observation is supported by other studies reporting that climate plays a pivotal role in the spread and seasonality of Lyme disease and some other tick-borne diseases.[14,15]

DOI: 10.1093/ajcp/aqz139

Testing by serology was strongly preferred by providers over PCR. The ratio was nearly 9:1 in babesiosis testing (serology 392,483 paired IgG-IgM tests, PCR 44,860 tests), and it reached 18:1 in both anapasmosis (serology 341,266 paired IgG-IgM, PCR 19,284) and ehrlichiosis (serology 489,043 paired IgG-IgM, PCR 26,830). Some of these serological tests might include both acute and convalescent sera in the same patient and the ratio would therefore be correspondingly smaller. However, at least at MGH, we observed that acute and convalescent sera are only rarely ordered and most patients receive only a single serological test.[4] In practice, serology remains the primary method of Lyme disease diagnosis because it is quick and readily available, especially when dealing with high numbers of samples; PCR plays an adjunctive role as it may not detect *B burgdorferi* DNA from blood in cases of active or chronic disease, and therefore its results should be correlated with serologic and epidemiologic data and clinical presentation of the patient.[16,17] Likewise, the reference standard for diagnosis of tick-borne rickettsial diseases (anapasmosis, ehrlichiosis, and RMSF) is the immunofluorescence assay using paired serum samples obtained soon after illness onset and 2 to 4 weeks later.[18]

In conclusion, testing for most NLTBIs in this study increased dramatically from 2010 through 2016 based on data from a large reference laboratory with testing throughout the United States. The number of positive cases, though not as stringent as criteria for public health reporting, generally exceeds that reported by CDC. Positive results mostly originated from a small number of states, reinforcing the geographic distribution for each NLTBI. Thus, this report provides an important complementary source of data to better understand trends in and spread of NLTBI. Clinicians should have high awareness of NLTBI, especially in states identified as having a relatively high level of identified cases.

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DOI: 10.1093/ajcp/aqz139