Lyme Disease

Laboratory Support of Diagnosis and Management

CLINICAL BACKGROUND

Lyme disease is by far the most common tick-borne disease in the United States. It is caused by the bacterium *Borrelia burgdorferi* and transmitted from the deer tick (*Ixodes scapularis* or *Ixodes pacificus*). Since 2008, approximately 30,000-40,000 cases of Lyme disease have been reported to the Centers for Disease Control and Prevention (CDC) every year, with most cases occurring during the summer months.[1] In 2019 there were about 35,000 confirmed and probable cases of Lyme disease based on reports submitted by healthcare providers[2]; however, the incidence is likely much higher based on insurance records.[3]

Lyme disease cases are heavily centered in New England and the Mid-Atlantic.[1,4] However, they are also found in Wisconsin and Minnesota and, to a lesser extent, other states in the Great Lakes and Pacific Coastal regions. Lyme disease is most common among children and middle-aged adults.[5]

The clinical presentation of Lyme disease is categorized as 1 of 3 stages: early localized, early disseminated, and late (**Table 1**).[5,8-14] In 70% to 80% of infected persons, early localized disease is characterized by erythema migrans (EM), a round skin lesion ≥5 cm in diameter that may appear in a "bulls-eye" pattern.[6] In the absence of EM, the differential diagnosis may include other tick-borne diseases such as *Borrelia miyamotoi* disease, which is often misdiagnosed as Lyme disease owing to overlapping symptoms.[7]

The first sign of early disseminated disease is often additional smaller lesions that may develop if Lyme disease is untreated; however, a recognized skin lesion does not always occur. Extracutaneous involvement in early disseminated disease can include the musculoskeletal, cardiac, or nervous systems.

In late-stage disease, Lyme carditis may overlap temporally with Lyme neuroborreliosis, a neurologic manifestation marked by symptoms such as cognitive impairment and memory difficulties.[17] About 10% to 15% of patients with untreated

Table 1. Lyme Disease: Stages, Symptoms, and Recommended Laboratory Testing[5,8-14]

Stage of disease	Symptom onset	Symptoms	Laboratory testing
Early localized	3 to 30 days after tick bite	• EM • Fever, myalgia, headache, nausea, fatigue	<2 weeks after the onset of symptoms: if skin lesions that are atypical for EM or a mixed infection (eg, Lyme disease and *B miyamotoi* disease) is clinically suspected, tick-borne PCR panel may be useful in the diagnosis of tick-borne disease 2 to 4 weeks after the onset of symptoms: acute (symptomatic) and convalescent (recovered) 2-tiered[a] IgG, IgM serology (if skin lesions that are atypical for EM)
Early disseminated	2 weeks to months after tick bite	• Atrioventricular heart block sometimes with myopericarditis • Migratory pain in joints, bone, and muscle • Secondary annular lesions • Malaise, fatigue	2 to 4 weeks after the onset of symptoms: acute and/or convalescent 2-tiered[a] IgG, IgM serology >4 weeks after the onset of symptoms: acute and/or convalescent 2-tiered[a] IgG serology
Late-stage	Months to years after tick bite	• Encephalopathy, polyneuropathy, lymphocytic meningitis • Prolonged, chronic arthritis • Lymphocytoma • Fatigue	Acute and/or convalescent 2-tiered[a] IgG serology in serum; consider serology and/or detection of *B burgdorferi* DNA in CSF or synovial fluid

CSF, cerebrospinal fluid; EM, erythema migrans; PCR, polymerase chain reaction.
[a] 2-tiered testing is a follow-up of a positive or equivocal ELISA with an immunoblot test (standard 2-tiered test) or a second ELISA (modified 2-tiered test), as recommended by the CDC and the Association of State and Territorial Public Health Laboratory Directors.[15,16]

Lyme disease will develop Lyme neuroborreliosis.[17] Lyme arthritis may also occur during late-stage disease and is the most common manifestation of Lyme disease months after initial tick exposure.[18] Left untreated, Lyme arthritis usually affects the knees over a period of several years.[18]

If initiated in the early stages of Lyme disease, treatment with appropriate antibiotics is usually effective.[9] Prophylaxis or serologic testing after a tick bite is usually not indicated in areas where less than 20% of ticks are infected; however, in areas where infected ticks are endemic, laboratory testing, including tick identification, is recommended.[9,19] This Clinical Focus provides information on appropriate test selection and interpretation in patients with suspected Lyme disease.

The information in the text and table is provided for informational purposes only and is not intended as medical advice. Test selection and interpretation, diagnosis, and patient management decisions should be based on the physician's education, clinical expertise, and assessment of the patient.

INDIVIDUALS SUITABLE FOR TESTING

- Symptomatic (**Table 1**) individuals with a history of exposure to a tick-endemic area

TEST AVAILABILITY

Laboratory tests that can help confirm the clinical diagnosis of Lyme disease include various serologic techniques and polymerase chain reaction (PCR)-based assays, as summarized in **Table 2**.

TEST SELECTION AND INTERPRETATION

A timeline of tick exposure in a tick-endemic area and symptoms of Lyme disease guide appropriate test selection.[1,9,19] The CDC recommends testing for IgM or IgG antibodies using 2-tiered tests: a standard 2-tiered test (STTT) or a modified 2-tiered test (MTTT).[15,16] It is reported that MTTT detects up to 30% more cases compared to STTT in patients with early Lyme disease.[21,22] PCR is recommended for some non-Lyme, tick-borne diseases (eg, *Borrelia miyamotoi* disease, Powassan virus infection, and anaplasmosis) that are included in Lyme disease differential diagnosis.[12,13,23-26] The sections below outline appropriate test selection based on the stage of disease along with characteristic test results.

Early localized Lyme disease

Diagnosis of early localized Lyme disease can sometimes be made on the basis of EM alone without laboratory testing.[9,20] The Infectious Diseases Society of America (IDSA) suggests that PCR methodology not be used for the diagnosis of Lyme disease.[19] However, in patients at less than 2 weeks after symptom onset, PCR may be helpful to identify non-Lyme tick-borne diseases if there is diagnostic uncertainty or if mixed infection is suspected.[11-13,23-26] Two-tiered testing should be used 2 to 4 weeks after the onset of symptoms; an increase in IgM titers may not be detected in specimens collected within 2 weeks following a tick bite.[9,27] IgM antibodies may be present within a few weeks of disease onset, whereas large increases in IgG titers are produced months later; thus, 2-tiered testing that is positive for IgM and negative for IgG indicates early infection.

Early disseminated Lyme disease

Two-tiered testing is recommended when clinical findings are suggestive of early disseminated Lyme disease (**Table 1**).[5,11] For specimens collected at 2 to 4 weeks after onset of symptoms, 2-tiered testing that is positive for IgM and negative for IgG indicates early infection, unless obtained on a specimen collected more than 1 month after onset of symptoms. If the specimen was collected more than 1 month after onset of symptoms, a positive IgM finding is more likely to represent a false-positive result unless IgG is also positive; vaccination or other diseases may also cause false-positive results. A positive IgG result by 2-tiered testing is required to confirm the diagnosis of early disseminated Lyme disease, but does not differentiate between active and past *B burgdorferi* infection.[11,28]

Negative serology results may indicate lack of infection or lack of seroconversion, which may occur if samples are collected too early after disease onset or when early antibiotic therapy blunts the antibody response. PCR-based assays can be useful in the workup of *B burgdorferi* infection if seroconversion has not yet occurred; these assays, however, are limited by low clinical sensitivity (18%).[29] Untreated patients who continue to be symptomatic but are seronegative for 6 to 8 weeks are unlikely to have Lyme disease, and a differential diagnosis should be considered.[19]

Late-stage Lyme disease

In patients with suspected Lyme disease that has been left untreated for months to years after a tick bite, symptoms that are characteristic of late-stage disease such as Lyme arthritis or Lyme neuroborreliosis can help guide diagnostic test selection. Detection of *Borrelia* DNA in synovial fluid, commonly from the knees, supports the diagnosis of Lyme arthritis (sensitivity, 78%; specificity, 100%).[18,29] A diagnosis of Lyme neuroborreliosis can be supported if *Borrelia* antibody or DNA are detected in cerebrospinal fluid (CSF). Antibody levels in CSF can be measured by ELISA or nephelometry and compared to control levels (ie, serum antibody or albumin) in a ratio defined as an antibody index; an elevated antibody index strongly supports a diagnosis of Lyme neuroborreliosis.[30] *Borrelia* DNA in CSF can be detected by PCR-based assays, which can support a diagnosis of Lyme neuroborreliosis; however, detection by PCR may be limited owing to low clinical sensitivity (38%).[31]

Table 2. Tests Available for Diagnosis and Management of Lyme Disease

Test code	Assay	Method	Clinical use
39209	*Borrelia burgdorferi* DNA, Qualitative Real-Time PCR, Miscellaneous[a,b]	Real-time PCR	Diagnose Lyme disease
93795	*Borrelia miyamotoi* DNA, Qualitative Real-Time PCR, Miscellaneous[a]	Real-time PCR	Diagnose *B miyamotoi* disease
39684	*Borrelia miyamotoi* Antibody (IgM, IgG), Immunoassay[a]	Immunoassay	Diagnose *B miyamotoi* disease
39219	*Borrelia* Species DNA, Real-Time PCR, with Reflexes, Blood[b,c]	Real-time PCR	Detect *Borrelia* spp DNA; diagnose Lyme disease or *B miyamotoi* disease
39218	*Borrelia* Species DNA, Real-Time PCR, with Reflexes, Synovial Fluid/CSF[b,c]	Real-time PCR	Detect *Borrelia* spp DNA; diagnose Lyme disease or *B miyamotoi* disease
6646	Lyme Disease (*Borrelia* spp) Antibody with Reflex to Blot (IgG, IgM)[c]	Immunoassay	Diagnose Lyme disease by standard 2-tiered test
39733	Lyme Disease Antibody with Reflex to Immunoassay (IgG, IgM)[c]	Immunoassay	Diagnose Lyme disease by modified 2-tiered test
34194	Lyme Disease Antibody Index for CNS Infection	ELISA; Nephelometry	Diagnose Lyme neuroborreliosis
29477	Lyme Disease Antibody (IgG), Immunoblot	Immunoblot	Confirm Lyme disease when ELISA results are positive or equivocal
8593	Lyme Disease Antibodies (IgG, IgM), Immunoblot		
15777	Lyme Disease (*Borrelia* spp) DNA, Qualitative Real-Time PCR, Blood[a]	Real-time PCR	Detect *Borrelia* spp DNA
15564	Lyme Disease (*Borrelia* spp) DNA, Qualitative Real-Time PCR, CSF/Synovial Fluid[a]	Real-time PCR	Diagnose Lyme neuroborreliosis or Lyme arthritis
15510	Lyme Disease (*Borrelia* spp) DNA, Qualitative Real-Time PCR, Tick[a]	Real-time PCR	Detect *B burgdorferi* in tick to assess risk of Lyme disease
90558	Tick ID with Reflex to Lyme Disease DNA, Real-Time PCR, Tick[c]	Microscopy; reflex to PCR	Identify tick and *B burgdorferi* to assess risk of tick-borne disease and assist with differential diagnosis
94322	Tick-borne Disease, Acute Molecular Panel[a,d] Includes *Anaplasma phagocytophilum* DNA, Qualitative Real-Time PCR (test code 17320); *Babesia microti* DNA, Real-Time PCR (test code 37314); *Borrelia miyamotoi* DNA, Real-Time PCR, Miscellaneous (test code 93795); *Ehrlichia chaffeensis* DNA, Real-Time PCR (test code 11353); Lyme Disease (*Borrelia* spp) DNA, Qualitative, Real-Time PCR, Blood (test code 15777)	Real-time PCR	Diagnose tick-borne diseases when selecting tests for individual pathogens is challenging owing to overlapping geographic distributions and clinical presentations of illness; especially useful to diagnose mixed infections
36942	Tick-borne Disease, Antibody Panel[a,d] Includes *Anaplasma phagocytophilum* Antibodies (IgG and IgM) (test code 34464); *Babesia duncani* (WA1) IgG Antibody, IFA (test code 17231); *Babesia microti* Antibodies (IgG, IgM) (test code 34300); Lyme Disease Ab with Reflex to Blot (IgG, IgM) (test code 6646); *Ehrlichia chaffeensis* (IgG, IgM) (test code 34271)	IFA	Diagnose tick-borne diseases when selecting tests for individual pathogens is challenging owing to substantial clinical overlap and co-infection

CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescence assay; PCR, polymerase chain reaction.

[a] This test was developed and its analytical performance characteristics have been determined by Quest Diagnostics. It has not been cleared or approved by the US Food and Drug Administration. This assay has been validated pursuant to the CLIA regulations and is used for clinical purposes.

[b] Please refer to the Quest Test Directory for your service area for test availability.

[c] Reflex tests are performed at an additional charge and are associated with an additional CPT code(s).

[d] Panel components may be ordered individually.

References

1. Lyme disease updates and new educational tools for clinicians. Centers for Disease Control and Prevention. Center for Preparedness and Response. May 20, 2021. Accessed May 24, 2021. https://emergency.cdc.gov/coca/ppt/2021/052021_Lyme_Disease_Slides.pdf
2. Lyme disease. Recent surveillance data. Centers for Disease Control and Prevention. Updated April 29, 2021. Accessed May 24, 2021. https://www.cdc.gov/lyme/datasurveillance/recent-surveillance-data.html
3. How many people get Lyme disease. Centers for Disease Control and Prevention. Updated January 13, 2021. Accessed July 8, 2021 https://www.cdc.gov/lyme/stats/humancases.html
4. Lyme disease maps: most recent year. Centers for Disease Control and Prevention. Updated April 29, 2021. Accessed May 26, 2021. https://www.cdc.gov/lyme/datasurveillance/maps-recent.html
5. Shapiro ED. Clinical practice. Lyme disease. *N Engl J Med.* 2014;370(18):1724-1731. doi:10.1056/NEJMcp1314325
6. Signs and symptoms of untreated Lyme disease. Centers for Disease Control and Prevention. Updated January 15, 2021. Accessed May 26, 2021. https://www.cdc.gov/lyme/signs_symptoms/index.html
7. Telford SR, Goethert HK, Molloy PJ, et al. Borrelia miyamotoi disease: neither Lyme disease nor relapsing fever. *Clin Lab Med.* 2015;35(4):867-882. doi:10.1016/j.cll.2015.08.002
8. Shapiro ED. Lyme disease. *N Engl J Med.* 2014;371(7):684. doi:10.1056/NEJMc1407264
9. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis.* 2006;43(9):1089-1134. doi:10.1086/508667
10. Schutzer SE, Berger BW, Krueger JG, et al. Atypical erythema migrans in patients with PCR-positive Lyme disease. *Emerg Infect Dis.* 2013;19(5):815-817. doi:10.3201/eid1905.120796
11. Tickborne diseases of the United States. Centers for Disease Control and Prevention. Updated October 1, 2020. Accessed May 26, 2021. https://www.cdc.gov/ticks/tickbornediseases/lyme.html
12. Lyme disease co-infection. National Institute of Allergy and Infectious Diseases. Updated November 16, 2018. Accessed July 16, 2021. https://www.niaid.nih.gov/diseases-conditions/lyme-disease-co-infection
13. Relapsing fever. B. miyamotoi. Centers for Disease Control and Prevention. Updated September 10, 2019. Accessed July 16, 2021. https://www.cdc.gov/relapsing-fever/miyamotoi/index.html
14. Emerging tickborne diseases: CDC public health grand rounds emerging tickborne diseases. Centers for Disease Control and Prevention. Updated March 21, 2017. Accessed July 16, 2021. https://www.cdc.gov/grand-rounds/pp/2017/20170321-tickborne-diseases.html
15. Notice to readers recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. Centers for Disease Control and Prevention. *MMWR Morb Mortal Wkly Rep.* 1995;44(31):590-591.
16. Mead P, Petersen J, Hinckley A. Updated CDC recommendation for serologic diagnosis of Lyme disease. *MMWR Morb Mortal Wkly Rep.* 2019;68(32):703. doi:10.15585/mmwr.mm6832a4
17. Hildenbrand P, Craven DE, Jones R, et al. Lyme neuroborreliosis: manifestations of a rapidly emerging zoonosis. *Am J Neuroradiol.* 2009;30(6):1079-1087. doi:10.3174/ajnr.A1579
18. Steere AC. Treatment of Lyme arthritis. *J Rheumatol.* 2019;46(8):871-873. doi:10.3899/jrheum.190320
19. Lantos PM, Rumbaugh J, Bockenstedt LK, et al. Clinical practice guidelines by the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR): 2020 guidelines for the prevention, diagnosis and treatment of Lyme disease. *Clin Infect Dis.* 2021;72(1):e1-e48. doi:10.1093/cid/ciaa1215
20. DePietropaolo DL, Powers JH, Gill JM, et al. Diagnosis of Lyme disease. *Am Fam Physician.* 2005;72(2):297-304.
21. B. burgdorferi IgG/IgM Test System [package insert]. Branchburg, NJ; ZEUS Scientific; 2020.
22. Branda JA, Body BA, Boyle J, et al. Advances in serodiagnostic testing for Lyme disease are at hand. *Clin Infect Dis.* 2017;66(7):1133-1139. doi:10.1093/cid/cix943
23. Powassan virus. Diagnostic testing. Centers for Disease Control and Prevention. Updated June 23, 2021. Accessed July 16, 2021. https://www.cdc.gov/powassan/diagnostic-testing.html
24. Anaplasmosis. Clinical and laboratory diagnosis. Centers for Disease Control and Prevention. Updated March 29, 2021. Accessed July 16, 2021. https://www.cdc.gov/anaplasmosis/healthcare-providers/clinical-lab-diagnosis.html
25. Telford SR, 3rd, Goethert HK, Molloy PJ, et al. Borrelia miyamotoi Disease: Neither Lyme Disease Nor Relapsing Fever. *Clin Lab Med.* 2015;35(4):867-882. doi:10.1016/j.cll.2015.08.002
26. Borrelia miyamotoi Disease. Centers for Disease Control and Prevention. Updated January 10, 2019. Accessed July 8, 2021. https://www.cdc.gov/ticks/tickbornediseases/borrelia-miyamotoi.html
27. Lyme disease. Diagnosis and testing. Centers for Disease Control and Prevention. Updated May 21, 2021. Accessed July 30, 2021. https://www.cdc.gov/lyme/diagnosistesting/index.html
28. Kalish RA, McHugh G, Granquist J, et al. Persistence of immunoglobulin M or immunoglobulin G antibody responses to Borrelia burgdorferi 10–20 years after active Lyme disease. *Clin Infect Dis.* 2001;33(6):780-785. doi:10.1086/322669
29. Aguero-Rosenfeld ME, Wang G, Schwartz I, et al. Diagnosis of Lyme borreliosis. *Clin Microbiol Rev.* 2005;18(3):484-509. doi:10.1128/cmr.18.3.484-509.2005
30. Blanc F, Jaulhac B, Fleury M, et al. Relevance of the antibody index to diagnose Lyme neuroborreliosis among seropositive patients. *Neurology*. 2007;69(10):953-958. doi:10.1212/01.wnl.0000269672.17807.e0
31. Nocton JJ, Bloom BJ, Rutledge BJ, et al. Detection of Borrelia burgdorferi DNA by polymerase chain reaction in cerebrospinal fluid in Lyme neuroborreliosis. *J Infect Dis.* 1996;174(3):623-627. doi:10.1093/infdis/174.3.623