MAYO CLINIC LABORATORIES

Test Definition: PBORB

Lyme Disease, Molecular Detection, PCR, Blood

Overview

Useful For

Supporting the diagnosis of Lyme disease in conjunction with serologic testing

This test **should not be used** to screen asymptomatic patients.

Testing Algorithm

For more information see Acute Tick-Borne Disease Testing Algorithm.

Special Instructions

- Acute Tick-Borne Disease Testing Algorithm

Method Name

Real-Time Polymerase Chain Reaction (PCR)/DNA Probe Hybridization

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Ordering Guidance

This assay does not detect *Borrelia miyamotoi.* If infection with this organism is suspected, order BMIPB / *Borrelia miyamotoi* Detection, PCR, Blood or BMIYC / *Borrelia miyamotoi* Detection, PCR, Spinal Fluid.

Specimen Required

Container/Tube: Lavender top (EDTA)

Specimen Volume: 1 mL

Collection Instructions: Send whole blood specimen in original tube. **Do not aliquot**.

Forms

If not ordering electronically, complete, print, and send a Microbiology Test Request (T244) with the specimen.

Specimen Minimum Volume

0.3 mL

Reject Due To

Gross hemolysis	OK

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Refrigerated (preferred)	7 days	
	Frozen	7 days	

Clinical & Interpretive

Clinical Information

Lyme disease is a multisystem and multistage tick-transmitted infection caused by spirochetal bacteria in the *Borrelia burgdorferi* sensu lato (Bbsl) complex.(1) Nearly all human infections are caused by 3 Bbsl species; *B burgdorferi* sensu stricto (hereafter referred to as *B burgdorferi*) is the primary cause of Lyme disease in North America, while *Borrelia afzelii* and *Borrelia garinii* are the primary causes of Lyme disease in Europe. In 2012, *Borrelia mayonii* was identified as a less common cause of Lyme disease in the upper Midwestern United States.(2,3) This organism has only been detected in patients with exposure to ticks in Minnesota and Wisconsin and has not been detected in over 10,000 specimens from patients in other states, including regions of the northeast where Lyme disease is endemic.

Lyme disease is the most commonly reported tick-borne infection in Europe and North America, causing an estimated 300,000 cases in the United States each year and 85,000 cases in Europe.(4,5) The clinical features of Lyme disease are broad and may be confused with various immune and inflammatory disorders. The classic presenting sign of early localized Lyme disease caused by *B burgdorferi* is erythema migrans (EM), which occurs in approximately 80% of individuals. Other early signs and symptoms include malaise, headache, fever, lymphadenopathy, and myalgia. Arthritis, neurological disease, and cardiac disease may be later stage manifestations. EM has also been seen in patients with *B mayonii* infection, but diffuse rashes are more commonly reported.(2) The chronic skin condition, acrodermatitis chronicum atrophicans, is also associated with *B afzelii* infection.

The presence of EM in the appropriate clinical setting is considered diagnostic for Lyme disease; no confirmatory laboratory testing is needed. In the absence of a characteristic EM lesion, serologic testing is the diagnostic method of choice for Lyme disease.(6) However, serology may not be positive until 1 to 2 weeks after onset of symptoms and may show decreased sensitivity for detection of infection with *B mayonii.* Therefore, detection of Bbsl DNA using polymerase chain reaction (PCR) may be a useful adjunct to serologic testing for detection of acute disease. PCR has shown utility for detection of *Borrelia* DNA from skin biopsies of Lyme-associated rashes and can be used to detect *Borrelia* DNA from synovial fluid and synovium biopsies. Less commonly, *Borrelia* DNA can be detected in cerebrospinal fluid and blood.(7) In general, blood is not the preferred source for detection of Bbsl DNA by PCR, although it may have increased utility for detection of *B mayonii*, due to the higher levels of observed peripheral spirochetemia with this organism.(2) Lyme PCR should always be performed in conjunction with US Food and Drug Administration approved serologic tests, and results should be correlated with serologic and epidemiologic data and clinical presentation of the patient.(8) The Mayo Clinic Lyme PCR test detects and differentiates the main causes of Lyme disease in North America (*B burgdorferi* and *B mayonii*) and Europe (*B afzelii* and *B garinii*).(2,7)

Reference Values

Negative

Reference values apply to all ages.

Interpretation

A positive result indicates the presence of DNA from *Borrelia burgdorferi*, *Borrelia mayonii*, *Borrelia afzelii,* or *Borrelia garinii,* the main agents of Lyme disease.

A negative result indicates the absence of detectable target DNA in the specimen. Due to the diagnostic sensitivity limitations of the polymerase chain reaction assay, a negative result does not preclude the presence of the organism or active Lyme disease.

Cautions

Serologic tests are recommended for diagnosis of Lyme disease. Polymerase chain reaction (PCR) may play an adjunctive role but may not detect *Borrelia burgdorferi* DNA from blood in cases of active or chronic disease. The presence of inhibitory substances may also cause a false-negative result. PCR test results should be used as an aid in diagnosis and not considered diagnostic by themselves. These results should be correlated with serologic and epidemiologic data and clinical presentation of the patient.

Concurrent infections with multiple tick-borne pathogens, including *Ehrlichia muris eauclairensis, Anaplasma phagocytophilum*, *Babesia microti,* and *Borrelia miyamotoi* (a relapsing-fever *Borrelia*) have been reported in the United States, and consideration should be given to testing for other pathogens, if clinically indicated.

This assay detects most members of the *B burgdorferi* sensu lato complex (Bbsl), including *Borrelia andersoni, Borrelia americana,* and *Borrelia bissettii*, which have been rarely detected in humans. Detection of DNA from these organisms would be reported as an atypical result and prompt additional laboratory testing to further identify the DNA present. The sensitivity of this assay for detecting these organisms has not been determined.

This assay also detects some members of the Bbsl complex that are not considered to be human pathogens but may be found in ticks and other animals. Therefore, this assay should not be used to test nonhuman specimens.

Supportive Data

The following validation data supports the use of this assay for clinical testing.

Analytical Sensitivity/Limit of Detection:
The lower limit of detection is approximately 300 to 1000 genomic copies/mL in cerebrospinal fluid, tissue, blood, and synovial fluid.

Accuracy/Diagnostic Sensitivity and Specificity:
Spiking studies of whole organism in whole blood (spiked near the approximate limit of detection) showed 100% recovery.

Analytical Specificity:
No polymerase chain reaction signal was obtained from the extracts of 22 bacterial, viral, parasitic, and fungal isolates that can cause symptoms similar to Lyme disease, including *Rickettsia rickettsii, Rickettsia typhi, Ehrlichia canis, Babesia*

microti, Plasmodium falciparum, Plasmodium vivax, Bartonella henselae, Bartonella quintana, herpes simplex virus, and *Toxoplasma gondii.* Relapsing fever borreliae (including *Borrelia miyamotoi*) are also not detected with this assay.

Precision:
Interassay precision was 100% and intra-assay precision was 100%.

Reference Range:
The reference range for this assay is negative. This assay is only to be used for patients with a clinical history and symptoms consistent with Lyme disease and must be interpreted in the context of serologic tests, which are the gold standard for diagnosis of Lyme disease.

Reportable Range:
This is a qualitative assay, and the results are reported as negative or positive for targeted *Borrelia burgdorferi*.

Clinical Reference

1. Stanek G, Wormser GP, Gray J, Strle F: Lyme borreliosis. Lancet. 2012 Feb;379(9814):461-473
2. Pritt BS, Mead PS, Johnson, DK, et al: Identification of a novel pathogenic *Borrelia* species causing Lyme borreliosis with unusually high levels of spirochetemia: a descriptive study. Lancet Infect Dis. 2016 May;16(5):556-564
3. Pritt BS, Respicio-Kingry LB, Sloan LM, et al: *Borrelia mayonii* sp. nov., a member of the *Borrelia burgdorferi* sensu lato complex, detected in patients and ticks in the upper midwestern United States. Int J Sys Evol Microbiol. 2016 Nov;66(11):4878-4880
4. Hinckley AF, Connally NP, Meek JI, et al: Lyme disease testing by large commercial laboratories in the United States. Clin Infect Dis. 2014 Sep 1;59(5):676-681
5. Lindgren E, Jaenson TGT: Lyme borreliosis in Europe: influences of climate and climate change, epidemiology, ecology and adaptation measures. Copenhagen, Denmark: World Health Organization; 2006
6. Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. MMWR Morb Mortal Wkly Rep. 1995 Aug;44(31):590-591
7. Babady NE, Sloan LM, Vetter EA, et al: Percent positive rate of Lyme real-time polymerase chain reaction in blood, cerebrospinal fluid, synovial fluid, and tissue. Diagn Microbiol Infect Dis. 2008 Dec;62(4):464-466
8. Centers for Disease Control and Prevention (CDC). Lyme disease--United States, 1995. MMWR Morb Mortal Wkly Rep. 1996 Jun;45(23):481-484

Performance

Method Description

Nucleic acid is extracted from clinical specimens using the automated MagNA Pure LC instrument system. The extract is then transferred wells of a 96-well plate for amplification. The LightCycler is an automated instrument that amplifies and monitors the development of target nucleic acid (amplicon) after each cycle of polymerase chain reaction (PCR). The DNA target for PCR assay is the 283-base pairs plasminogen-binding protein gene *(OppA2),* which is present at a frequency of 1 copy per organism in all 4 confirmed pathogenic species of the *Borrelia burgdorferi* sensu lato genogroup (*B burgdorferi* sensu stricto, *Borrelia afzelii*, *Borrelia garinii,* and *Borrelia mayonii*). A specific base pair DNA target

sequence is amplified by PCR. The detection of amplicon is based on fluorescence resonance energy transfer, which utilizes 1 hybridization probe with a donor fluorophore, fluorescein, at the 3' end, and a second hybridization probe with an acceptor fluorophore, LC-Red 610, at the 5' end. When the target amplicon is present, the LC-Red 610 emits a measurable and quantifiable light signal at a specific wavelength. Presence of the specific organism nucleic acid may be confirmed by performing a melting curve analysis of the amplicon. Using features of the melting curve analysis, the assay primers and specific hybridization probes are able to detect and differentiate *B burgdorferi* sensu stricto from *B mayonii, B afzelii*, and *B garinii,* although the melting curve analysis cannot differentiate between *B afzelii* and *B garinii*. Each assay run can be completed within 60 minutes.(Unpublished Mayo method)

PDF Report
No

Day(s) Performed
June through November: Monday through Saturday
December through May: Monday through Friday

Report Available
1 to 4 days

Specimen Retention Time
1 week

Performing Laboratory Location
Rochester

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
87476
87798 x 2
87999 (if appropriate for government payers)

LOINC® Information

Test Definition: PBORB

Lyme Disease, Molecular Detection, PCR, Blood

Test ID	Test Order Name	Order LOINC® Value
PBORB	Lyme Disease PCR, B	90892-1

Result ID	Test Result Name	Result LOINC® Value
56080	B. burgdorferi PCR, B	94247-4
38290	B. mayonii PCR, B	94248-2
38291	B. garinii/B. afzelii PCR, B	94249-0
38340	Lyme Disease PCR Comment	59464-8