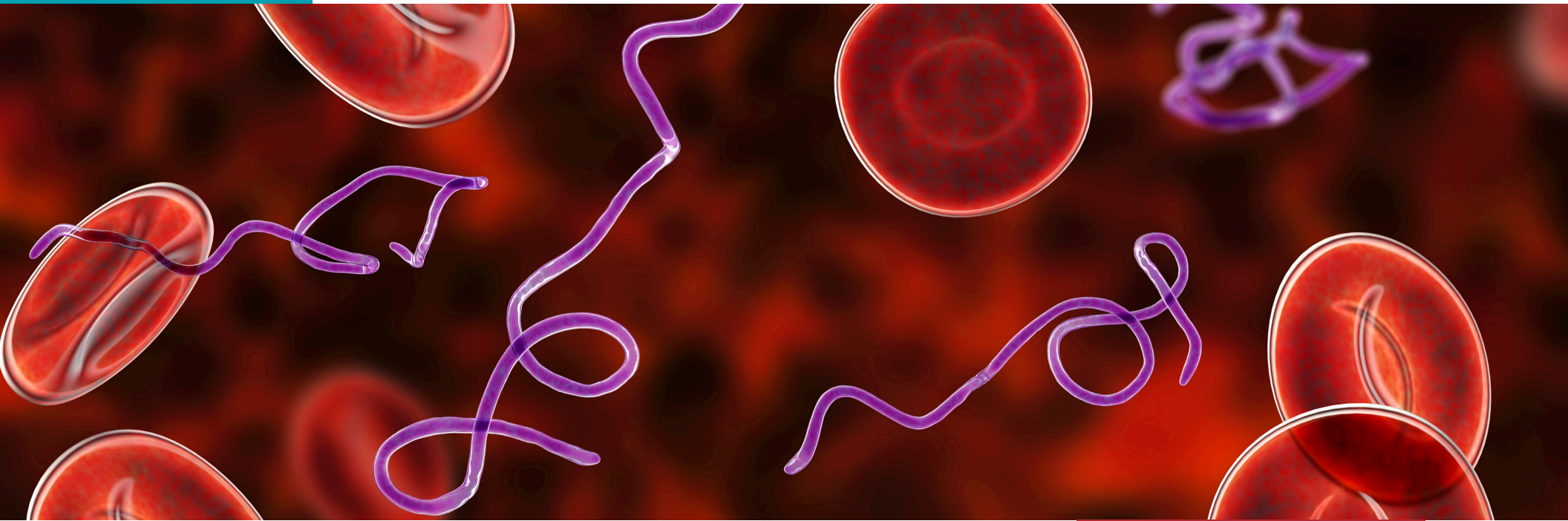


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Suggested Reporting Language, Interpretation and Guidance Regarding Lyme Disease Serologic Test Results



MAY 2021

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BACKGROUND

Lyme disease, predominantly caused by *Borrelia burgdorferi* (also referred to as *Borrelia burgdorferi*),¹ is currently the most common tick-borne illness in North America. In 2017, over 40,000 confirmed or probable Lyme disease cases were reported to the US Centers for Disease Control and Prevention (CDC). The true incidence of this disease, however, is estimated to be 8-10 times higher, with greater than 5 million diagnostic tests being performed every year.²⁻⁶ Diagnosis of Lyme disease is reliant on both a clinical suspicion of disease and on appropriate utilization of diagnostic tests, including serologic assays for detection of an immune response to infection with *B. burgdorferi*. The recommended serologic testing algorithms involve multiple tests, and correct result interpretation depends on the timing of testing relative to symptom onset and disease manifestation.

Differences in language and formatting of diagnostic test reports used by clinical laboratories can lead to misinterpretation of results and confusion by both providers and patients, which may lead to poor patient health outcome or misdiagnosis. This document was created by the Association for Public Health Laboratories (APHL) and an associated workgroup comprised of subject matter experts from public health agencies, and public health and clinical laboratories, to address the proper interpretation of serologic testing for *B. burgdorferi*, as well as to identify best practices for reporting results to clinicians, public health agencies and patients. The reporting guidance outlined in this document is only suggestive and may need to be adapted or modified depending on local factors or advances in diagnostic technology.

INTRODUCTION

Lyme disease is a bacterial disease, transmitted through the bite of *Ixodes* spp. or blacklegged ticks. The identification of *B. burgdorferi*⁷ as the causative agent of Lyme disease in 1982 initiated the development of multiple tests by assay manufacturers and clinical laboratories. Recently, *Borrelia mayonii* has also been identified as a causative agent of Lyme disease in North America, although it is currently localized to Wisconsin and Minnesota.⁸ *B. burgdorferi* bacteria only enter the bloodstream transiently, and direct detection methods such as culture or PCR are typically insensitive for most specimen sources (e.g., blood, spinal fluid, etc.).^{9, 10} Due to this limitation, diagnostic testing for Lyme disease relies on indirect detection of infection by measuring a patient's antibody response to the spirochete.

The initial years of Lyme disease diagnostic test development generated a variety of serologic assays, which demonstrated lack of inter- and intra-assay precision and accuracy, necessitating standardization. To remediate this issue, the Second National Conference on the Serologic Diagnosis of Lyme Disease (Dearborn, Michigan, USA) was convened to review the available evidence and to generate a standard testing strategy.¹¹ During the evaluation of the variety of testing platforms, it was determined that no single serologic test for Lyme disease was sufficiently sensitive and specific on its own. A standard two-tiered testing (STTT) method for serologic diagnosis of Lyme disease was agreed upon to maximize clinical utility. All US Food and Drug Administration (FDA) cleared tests were based on the STTT method until 2019, when the FDA cleared assays for use in a modified two-tiered testing (MTTT) method, as an alternative serologic approach for detection of Lyme disease.¹² These serologic approaches for the diagnosis of Lyme disease, including recommended result reporting and interpretative guidance, are summarized here.

Clinical Description/Testing Guidelines

Lyme disease is characterized by protean manifestations, including potential development of dermatologic, rheumatologic, neurologic and cardiac abnormalities. The most common clinical marker for early Lyme disease is erythema migrans (EM), which occurs in 60%-80% of patients. EM is defined as a skin lesion that typically begins as a red macule or papule and expands over a period of days to weeks to form a large (reaching diameters of up to 30cm) round lesion, often with partial central clearing. Secondary lesions also may occur.¹³ Annular erythematous lesions occurring within several hours of a tick bite typically represent hypersensitivity reactions and do not qualify as EM. Patients who have a typical EM lesion, identified by a physician, and who live in or have traveled to a Lyme-endemic area can be diagnosed with acute Lyme disease without laboratory diagnostic support as EM lesions are considered pathognomonic of infection. Importantly, diagnostic testing of patients presenting with EM lesions is not

recommended due to insensitivity of serologic assays during this acute stage of infection.⁵

Later signs and symptoms of Lyme disease may appear days to months after a tick bite. Symptoms of early dissemination can manifest as additional EM rashes on other areas of the body. Although less frequent, dissemination at any time following infection can result in neuroinvasive Lyme disease, including lymphocytic meningitis, facial nerve palsy or radiculoneuropathy. Lyme carditis, characterized by atrioventricular (AV) heart block, may also occur and, left untreated, can result in sudden death. Hallmarks of later stages of disease include arthritis accompanied by severe joint pain and swelling, particularly in the knees and other large joints. Serologic testing by STTT has shown to be both sensitive (>87%) and specific (99%) for manifestations of disseminated Lyme disease¹¹ and can provide strong support for diagnosis.

It is important for healthcare providers to order Lyme disease testing only when there is existing clinical and epidemiological support for a diagnosis.¹⁴ Lyme disease should be considered based on the presence of typical signs and symptoms of infection in patients with a history of possible exposure to infected blacklegged ticks. Serologic tests using FDA-cleared methods and recommended interpretive criteria should be used.¹¹ Existing tests for Lyme disease are sensitive and specific if performed as recommended at appropriate times post-infection or for manifestations of disseminated disease. False positive tests do occur, primarily in cases with a low prior probability of Lyme disease, such as for patients without likely exposure to infected blacklegged ticks (e.g., exposure to ticks only in areas of low incidence of Lyme disease).¹⁵ Similarly, false negative results may occur in patients who are tested too soon following infection, at which point the patient's serologic response has not developed and is therefore not yet detectable. Testing of patients who do not have symptoms typical of Lyme disease or who have not had the potential for exposure to ticks in a Lyme-endemic region, is strongly discouraged. Just as it is important to correctly diagnose Lyme disease in a patient who has the disease, it is equally important to avoid misdiagnosis of Lyme disease and unnecessary treatment when the true cause of illness is something else.

The intent of this document is to clearly outline scenarios when Lyme disease testing is necessary, as well as outline proper application of the STTT or MTTT for testing of human samples. Additionally, recommended standard reporting language for the STTT and MTTT is provided, with an emphasis on clear and concise interpretations to provide clarity for clinicians, laboratorians and patients.

DESCRIPTION OF LYME DISEASE STANDARD TWO-TIERED TESTING AND SUGGESTED REPORTING AND INTERPRETATION TABLE

The STTT begins with an immunoassay detecting IgM or IgG antibodies to *B. burgdorferi*. Either an enzyme immune assay (immunoassay) or, newer generation (e.g., lateral flow, fluorescence and chemiluminescence) assays available on other platforms can be performed. If the immunoassay(s) are negative, no further testing is necessary. If the total IgM/IgG immunoassay, or either one or both of the first tier IgM and IgG immunoassays are positive or equivocal, reflex testing by immunoblot is required. For samples collected from patients with symptoms lasting 30 days or less, both IgM and IgG specific anti-*B. burgdorferi* immunoblots should be performed and interpreted to guide clinical decisions. For samples collected over 30 days post symptom onset, only the anti-*B. burgdorferi* IgG immunoblot should be performed or interpreted. For detailed information regarding result interpretation, please see Table 1a (total IgM/IgG immunoassay) or Table 1b (separate IgM and IgG immunoassays).

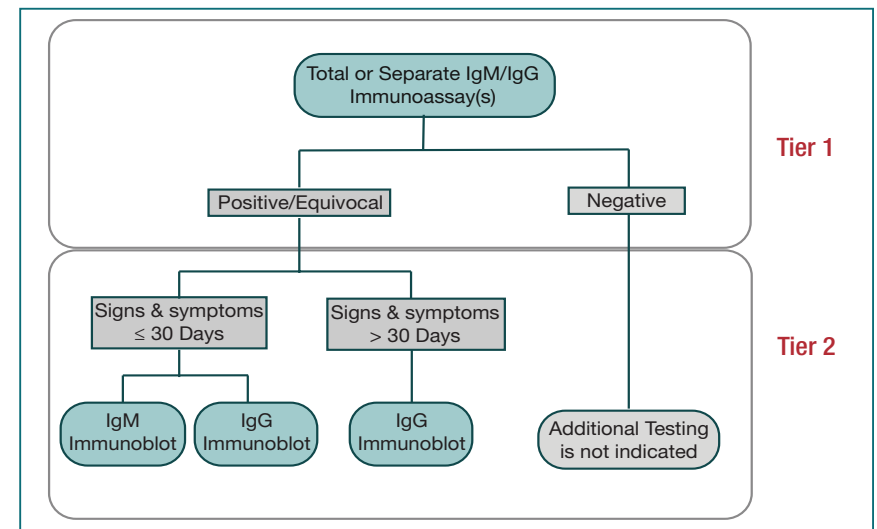


Figure 1: Standard Two-Tiered Testing (STTT)

Table 1a. Suggested Guidance for Reporting Results from the Standard Two-Tiered Lyme Disease Serologic Testing Using a Total Ig First Tier Assay

Test Sequence			Interpretation for Laboratories	Interpretation for Providers	Comments / Further Actions (may be included on the laboratory report)
Tier 1	Tier 2a	Tier 2b			
Total Ig Immunoassay	IgM Immunoblot ^{a, b}	IgG Immunoblot ^c			
Negative	Testing Not Indicated ^d	Testing Not Indicated ^d	Negative for antibodies to <i>B. burgdorferi</i> (Lyme disease).	No laboratory evidence of infection with <i>B. burgdorferi</i> (Lyme disease).	Negative results may occur in patients recently infected (≤ 14 days) with <i>B. burgdorferi</i> . If recent infection is suspected, repeat testing on a new sample collected in 7-14 days is recommended.
Positive/ Equivocal	Negative	Negative	Antibodies to <i>B. burgdorferi</i> (Lyme disease) not confirmed.	No laboratory evidence of infection with <i>B. burgdorferi</i> (Lyme disease).	Negative results may occur in patients recently infected (≤ 14 days) with <i>B. burgdorferi</i> . If recent infection is suspected, repeat testing on a new sample collected in 7-14 days is recommended.
		IgG Detected Against: (list) ^e			
Positive/ Equivocal	Positive	Negative	IgM-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Results are consistent with acute or recent infection with <i>B. burgdorferi</i> (Lyme disease).	IgM immunoblot results should only be considered as indicative of recent infection in patients presenting within 30 days of symptom onset. Consideration of IgM immunoblot results in patients with symptoms lasting >30 days is discouraged due to the risk of false positive IgM immunoblot results or prolonged IgM seropositivity following disease resolution. Testing of a new specimen collected in 7-14 days to demonstrate IgG seroconversion may be considered to confirm infection.
	IgM Detected Against: (list)	IgM Detected Against: (list) ^e			
Positive/ Equivocal	Negative	Positive	IgG-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Results are consistent with <i>B. burgdorferi</i> (Lyme disease) infection in the recent or remote past. IgG-class antibodies may remain detectable for months to years following resolution of infection.	Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed.
		IgG Detected Against: (list)			
Positive/ Equivocal	Positive	Positive	IgM- and IgG-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Results are consistent with <i>B. burgdorferi</i> infection (Lyme disease) in the recent or remote past. Antibodies may remain detectable for months to years following resolution of infection.	Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed.
	IgG Detected Against: (list)	IgG Detected Against: (list)			

a Immunoblots for IgM antibodies to *B. burgdorferi* are interpreted as “negative” if <2 *B. burgdorferi*-specific proteins are detected. Conversely, if ≥ 2 out of a possible 3 *B. burgdorferi*-specific proteins are detected, the immunoblot is interpreted as “positive” for IgM-class antibodies to *B. burgdorferi*. The *B. burgdorferi*-specific proteins that may be detected include: p23, p39, p41.

b Testing for IgM antibodies to *B. burgdorferi* is not indicated in patients presenting >30 days post-symptom onset.

c Immunoblots for IgG antibodies to *B. burgdorferi* are interpreted as “negative” if <5 *B. burgdorferi*-specific proteins are detected. Conversely, if ≥ 5 out of a possible 10 *B. burgdorferi*-specific proteins are detected, the immunoblot is interpreted as “positive” for IgG-class antibodies to *B. burgdorferi*. The *B. burgdorferi*-specific proteins that may be detected include: p18, p23, p28, p30, p39, p41, p45, p58, p66, p93.

d In accordance with the current standard two-tiered testing algorithm, testing by the IgM and IgG blots is not indicated due to negative initial screening immunoassay.

e Reporting of individual IgG bands is recommended even when the overall test result is negative, because some physicians may use this information to guide decisions about treatment or repeat testing.

Table 1b. Suggested Guidance for Reporting Results from the Standard Two-Tiered Lyme Disease Serologic Testing Algorithm Using Separated IgG and IgM First Tier Assays

Test Sequence			Interpretation for Laboratories	Interpretation for Providers	Comments / Further Actions (may be included on the laboratory report)
Tier 1	Tier 2a	Tier 2b			
Total Ig Immunoassay	IgM Immunoblot ^{a, b}	IgG Immunoblot ^c			
Tier 1 IgM and IgG Immunoassay results in concordance					
Negative	Testing Not Indicated ^d	Testing Not Indicated ^d	Negative for antibodies to <i>B. burgdorferi</i> (Lyme disease).	No laboratory evidence of infection with <i>B. burgdorferi</i> (Lyme disease).	Negative results may occur in patients recently infected (≤ 14 days) with <i>B. burgdorferi</i> . If recent infection is suspected, repeat testing on a new sample collected in 7–14 days is recommended.
Positive/ Equivocal by both IgM and IgG assays	Negative	Negative	Antibodies to <i>B. burgdorferi</i> (Lyme disease) not confirmed.	No laboratory evidence of infection with <i>B. burgdorferi</i> (Lyme disease).	Negative results may occur in patients recently infected (≤ 14 days) with <i>B. burgdorferi</i> . If recent infection is suspected, repeat testing on a new sample collected in 7–14 days is recommended.
		IgG Detected Against: (list) ^e			
Positive/ Equivocal by both IgM and IgG assays	Positive	Negative	IgM-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Results are consistent with acute or recent infection with <i>B. burgdorferi</i> (Lyme disease).	IgM immunoblot results should only be considered as indicative of recent infection in patients presenting within 30 days of symptom onset. Consideration of IgM immunoblot results in patients with symptoms lasting >30 days is discouraged due to the risk of false positive IgM immunoblot results or prolonged IgM seropositivity following disease resolution. Testing of a new specimen collected in 7–14 days to demonstrate IgG seroconversion may be considered to confirm infection.
	IgM Detected Against: (list)	IgG Detected Against: (list) ^e			
Positive/ Equivocal by both IgM and IgG assays	Negative	Positive	IgG-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Results are consistent with <i>B. burgdorferi</i> (Lyme disease) infection in the recent or remote past. IgG-class antibodies may remain detectable for months to years following resolution of infection.	Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed.
		IgG Detected Against: (list)			
Positive/ Equivocal by both IgM and IgG assays	Positive	Positive	IgM- and IgG-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Results are consistent with <i>B. burgdorferi</i> infection (Lyme disease) in the recent or remote past. Antibodies may remain detectable for months to years following resolution of infection.	Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed.
	IgM Detected Against: (list)	IgG Detected Against: (list)			
Tier 1 Discordant IgM and IgG Immunoassay results					
IgM Positive/ Equivocal IgG Negative	Negative	Not Indicated or Negative	Negative for antibodies to <i>B. burgdorferi</i> (Lyme disease).	No laboratory evidence of infection with <i>B. burgdorferi</i> (Lyme disease).	Negative results may occur in patients recently infected (≤ 14 days) with <i>B. burgdorferi</i> . If recent infection is suspected, repeat testing on a new sample collected in 7–14 days is recommended.

Test Sequence			Interpretation for Laboratories	Interpretation for Providers	Comments / Further Actions (may be included on the laboratory report)
Tier 1	Tier 2a	Tier 2b			
Total Ig Immunoassay	IgM Immunoblot ^{a, b}	IgG Immunoblot ^c			
IgM Positive/ Equivocal IgG Negative	Positive	Not Indicated or If performed, results should not be considered for clinical care.	IgM-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Results are consistent with acute or recent infection with <i>B. burgdorferi</i> (Lyme disease).	IgM immunoblot results should only be considered as indicative of recent infection in patients presenting within 30 days of symptom onset. Consideration of IgM immunoblot results in patients with symptoms lasting >30 days is discouraged due to the risk of false positive IgM immunoblot results or prolonged IgM seropositivity following disease resolution. Testing of a new specimen collected in 7–14 days to demonstrate IgG seroconversion may be considered to confirm infection.
	IgM Detected Against: (list)				
IgM Negative/ Not performed IgG Positive/ Equivocal	Not Indicated or Negative	Negative	Negative for antibodies to <i>B. burgdorferi</i> (Lyme disease).	No laboratory evidence of infection with <i>B. burgdorferi</i> (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with <i>B. burgdorferi</i> . If recent infection is suspected, repeat testing on a new sample collected in 7-14 days is recommended.
		IgG Detected Against: (list) ^e			
IgM Negative/ Not performed IgG Positive/ Equivocal	Not Indicated or If performed, results should not be considered for clinical care.	Positive	IgG-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Results are consistent with <i>B. burgdorferi</i> (Lyme disease) infection in the recent or remote past. IgG-class antibodies may remain detectable for months to years following resolution of infection.	Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed.
		IgG Detected Against: (list)			
Tier 1 & 2 Discordant IgM and IgG Results					
IgM Positive/ Equivocal IgG Negative	Negative	Positive	Inconclusive	Repeat testing using the standard two-tiered or modified testing algorithm for Lyme disease is recommended. ^f	Consider further testing or alternate diagnosis.
		IgG Detected Against: (list)			
IgM Negative/ Not performed IgG Positive/ Equivocal	Positive	Negative	Inconclusive	Repeat testing using the standard or modified two-tiered testing algorithm for Lyme disease is recommended. ^f	Consider further testing or alternate diagnosis.

a Immunoblots for IgM antibodies to *B. burgdorferi* are interpreted as “negative” if <2 *B. burgdorferi*-specific proteins are detected. Conversely, if ≥2 out of a possible 3 *B. burgdorferi*-specific proteins are detected, the immunoblot is interpreted as “positive” for IgM-class antibodies to *B. burgdorferi*. The *B. burgdorferi*-specific proteins that may be detected include: p23, p39, p41.

b Testing for IgM antibodies to *B. burgdorferi* is not indicated in patients presenting >30 days post-symptom onset.

c Immunoblots for IgG antibodies to *B. burgdorferi* are interpreted as “negative” if <5 *B. burgdorferi*-specific proteins are detected. Conversely, if ≥ 5 out of a possible 10 *B. burgdorferi*-specific proteins are detected, the immunoblot is interpreted as “positive” for IgG-class antibodies to *B. burgdorferi*. The *B. burgdorferi*-specific proteins that may be detected include: p18, p23, p28, p30, p39, p41, p45, p58, p66, p93.

d In accordance with the current standard two-tiered testing algorithm, testing by the IgM and IgG blots is not indicated due to negative initial screening immunoassay.

e Reporting of individual IgG bands is recommended even when the overall test result is negative, because some physicians may use this information to guide decisions about treatment or repeat testing after more time.

f <https://www.cdc.gov/lyme/diagnosistesting/index.html>

DESCRIPTION OF LYME DISEASE STANDARD TWO-TIERED TESTING SPECIAL CIRCUMSTANCES TABLE

Testing for IgM or IgG-class antibodies to *B. burgdorferi* solely by immunoblot, without a prior positive or equivocal first-tier immunoassay, is strongly discouraged due to an increased frequency of false-positive results. However, some regional or national laboratories offer *B. burgdorferi* IgM/IgG immunoblot testing alone, without an up-front *B. burgdorferi* immunoassay, to be ordered by laboratories that only offer first-tier *B. burgdorferi* testing. Reference laboratories that offer a *B. burgdorferi* IgM/IgG immunoblot only orderable test assume that samples submitted for this test have screened positive or equivocal by a first-tier *B. burgdorferi* immunoassay. See Table 2 for suggested guidance for interpreting results under these special circumstances.

Table 2. Suggested Guidance for Special Circumstances (Tier 2 test performed with unknown Tier 1 results)

Test Sequence			Algorithm Interpretations	Interpretation for Laboratory Report	Comments / Further Actions (may be included on the laboratory report)
Tier 1	Tier 2a	Tier 2b			
Total Ig Immunoassay	IgM Immunoblot ^{a, b}	IgG Immunoblot ^c			
Unknown (Assumed Positive/ Equivocal)	Negative	Negative	Antibodies to <i>B. burgdorferi</i> (Lyme disease) not confirmed.	No laboratory evidence of infection with <i>B. burgdorferi</i> (Lyme disease).	Negative results may occur in patients recently infected (≤ 14 days) with <i>B. burgdorferi</i> . If recent infection is suspected, repeat testing of a new sample collected in 7-14 days is recommended using the two-tiered testing algorithm.
		IgG Detected Against: (list) ^d			
Unknown (Assumed Positive/ Equivocal)	Positive	Negative	IgM-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Interpretation of these results is only accurate if an initial <i>B. burgdorferi</i> immunoassay was positive or equivocal.	IgM immunoblot results should only be considered as indicative of recent infection in patients within 30 days of symptom onset. Consideration of IgM immunoblot results in patients with >30 days of symptoms is discouraged due to the risk of false positive IgM immunoblot results or prolonged IgM seropositivity following disease resolution. Testing of a new specimen collected in 7–14 days to demonstrate IgG seroconversion may be considered to confirm infection.
	IgM Detected Against: (list)	IgG Detected Against: (list) ^d		Results are consistent with acute or recent infection with <i>B. burgdorferi</i> (Lyme disease).	
Unknown (Assumed Positive/ Equivocal)	Negative	Positive	IgG-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Interpretation of these results is only accurate if an initial <i>B. burgdorferi</i> immunoassay was positive or equivocal. Results are consistent with <i>B. burgdorferi</i> (Lyme disease) infection in the recent or remote past. IgG-class antibodies may remain detectable for months to years following resolution of infection.	Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed.
		IgG Detected Against: (list)			
Unknown (Assumed Positive/ Equivocal)	Positive	Positive	IgM- and IgG-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Interpretation of these results is only accurate if an initial <i>B. burgdorferi</i> immunoassay was positive or equivocal. Results are consistent with <i>B. burgdorferi</i> (Lyme disease) infection in the recent or remote past. Antibodies may remain detectable for months to years following resolution of infection.	Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed.
	IgM Detected Against: (list)	IgG Detected Against: (list)			

a Immunoblots for IgM antibodies to *B. burgdorferi* are interpreted as “negative” if <2 *B. burgdorferi*-specific proteins are detected. Conversely, if ≥ 2 out of a possible 3 *B. burgdorferi*-specific proteins are detected, the immunoblot is interpreted as “positive” for IgM-class antibodies to *B. burgdorferi*. The *B. burgdorferi*-specific proteins that may be detected include: p23, p39, p41.

b Testing for IgM antibodies to *B. burgdorferi* is not indicated in patients presenting >30 days post-symptom onset.

c Immunoblots for IgG antibodies to *B. burgdorferi* are interpreted as “negative” if <5 *B. burgdorferi*-specific proteins are detected. Conversely, if ≥ 5 out of a possible 10 *B. burgdorferi*-specific proteins are detected, the immunoblot is interpreted as “positive” for IgG-class antibodies to *B. burgdorferi*. The *B. burgdorferi*-specific proteins that may be detected include: p18, p23, p28, p30, p39, p41, p45, p58, p66, p93

d Reporting of individual IgG bands is recommended even when the overall test result is negative, because some physicians may use this information to guide decisions about treatment or repeat testing.

DESCRIPTION OF LYME DISEASE MODIFIED TWO-TIERED TESTING AND SUGGESTED REPORTING AND INTERPRETATION TABLE

The MTTT method utilizes two immunoassays, based on multiple *B. burgdorferi* antigens, that have been cleared by FDA for this use. The MTTT begins with an immunoassay detecting antibodies to *B. burgdorferi*. Samples negative by this first tier test do not require further testing. If the total IgM/IgG immunoassay is positive or equivocal, reflex testing by a second immunoassay is required. The second immunoassay may be either total IgM/IgG (Figure 2 and Table 3) or separated IgM and IgG (Figure 3 and Table 4).

Figure 2: Modified Two-Tiered Testing (MTTT) 1 – Two Total IgM/IgG immunoassay

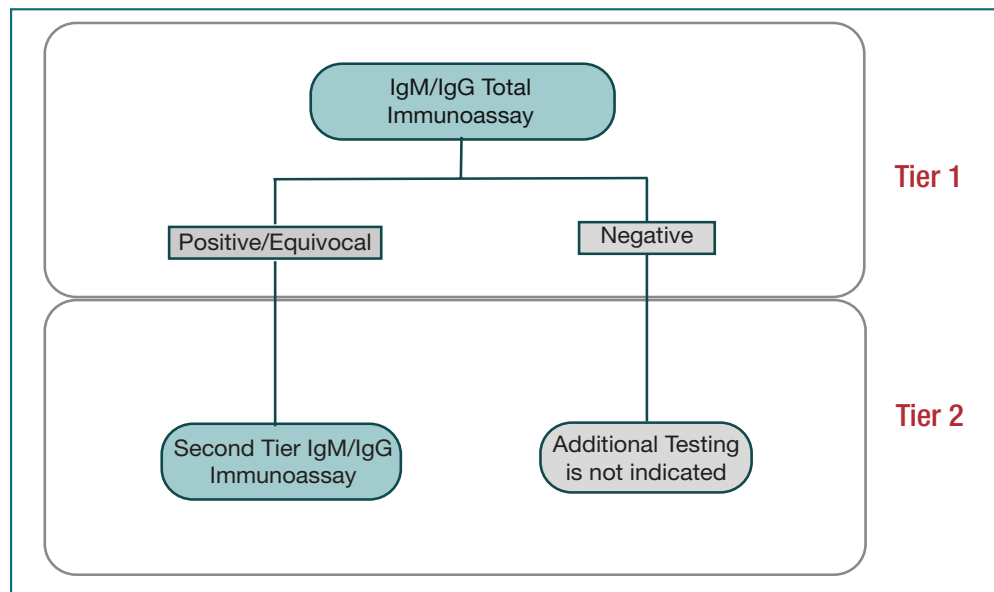


Table 3. Suggested Guidance for Reporting Results from the Modified Two-Tiered Lyme Disease Serologic Testing Algorithm Using Two *B. burgdorferi* IgM/IgG Immunoassays^a

Test Sequence		Interpretation for Laboratories	Interpretation for Providers	Comments / Further Actions (may be included on the laboratory report)
Tier 1	Tier 2			
<i>IgM/IgG Total Immunoassay</i>	<i>Whole Cell Antigen IgM/IgG Total Immunoassay</i>			
Negative	Testing Not Indicated	Negative for antibodies to <i>B. burgdorferi</i> (Lyme disease).	No laboratory evidence of infection with <i>B. burgdorferi</i> (Lyme disease).	Negative results may occur in patients recently infected (≤ 14 days) with <i>B. burgdorferi</i> . If recent infection is suspected, repeat testing on a new sample collected in 7–14 days is recommended.
Positive/ Equivocal	Negative	Antibodies to <i>B. burgdorferi</i> (Lyme disease) not confirmed.	No laboratory evidence of infection with <i>B. burgdorferi</i> (Lyme disease).	Negative results may occur in patients recently infected (≤ 14 days) with <i>B. burgdorferi</i> . If recent infection is suspected, repeat testing on a new sample collected in 7–14 days is recommended.
Positive/ Equivocal	Positive/Equivocal	IgM- or IgG-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected. Specific antibody class detected cannot be determined.	Results are consistent with <i>B. burgdorferi</i> infection (Lyme disease) in the recent or remote past. Antibodies may remain detectable for months to years following resolution of infection.	Timing of infection (acute/recent vs. past) cannot be determined by these assays. Clinical correlation is required. Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed. If both tests are equivocal consider repeat testing in 7–14 days if clinically warranted.

^a Only tests cleared by FDA for this intended purpose should be used

Figure 3: Modified Two-Tiered Testing Algorithm (MTTT) 2 – Separate IgM and IgG Second Tier immunoassays

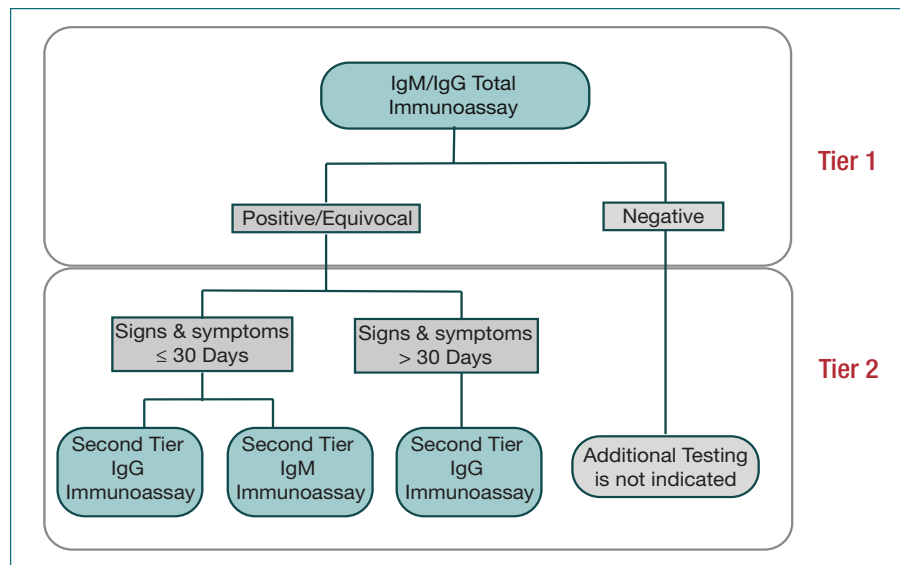


Table 4. Suggested Guidance for Reporting Results from the Modified Two-Tiered Lyme Disease Serologic Testing Algorithm Using Separate *B. burgdorferi* IgM and IgG Second Tier immunoassays^a

Test Sequence			Interpretation for Laboratories	Interpretation for Providers	Comments / Further Actions (may be included on the laboratory report)
Tier 1	Tier 2a	Tier 2b			
<i>VlsE/pepC10</i> IgM/IgG Total Immunoassay	<i>Whole Cell</i> Antigen IgM Immunoassay	<i>Whole Cell</i> Antigen IgG Immunoassay			
Negative	Testing Not Indicated	Testing Not Indicated	Negative for antibodies to <i>B. burgdorferi</i> (Lyme disease).	No laboratory evidence of infection with <i>B. burgdorferi</i> (Lyme disease).	Negative results may occur in patients recently infected (≤ 14 days) with <i>B. burgdorferi</i> . If recent infection is suspected, repeat testing on a new sample collected in 7–14 days is recommended.
Positive/ Equivocal	Negative	Negative	Antibodies to <i>B. burgdorferi</i> (Lyme disease) not confirmed.	No laboratory evidence of infection with <i>B. burgdorferi</i> (Lyme disease).	Negative results may occur in patients recently infected (≤ 14 days) with <i>B. burgdorferi</i> . If recent infection is suspected, repeat testing on a new sample collected in 7–14 days is recommended.
Positive/ Equivocal	Positive/ Equivocal	Negative	IgM-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Results are consistent with acute or recent infection with <i>B. burgdorferi</i> (Lyme disease).	IgM immunoassay results should only be considered as indicative of recent infections in patients presenting within 30 days of symptom onset. Consideration of IgM immunoblot results in patients with symptoms lasting >30 days is discouraged due to the risk of false positive IgM immunoblot results or prolonged IgM seropositivity following disease resolution. Testing of a new specimen collected in 7–14 days to demonstrate IgG seroconversion may be considered to confirm infection. If both tests are equivocal consider repeat testing in 7–14 days if clinically warranted.
Positive/ Equivocal	Negative	Positive/ Equivocal	IgG-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Results are consistent with <i>B. burgdorferi</i> (Lyme disease) infection in the recent or remote past. IgG-class antibodies may remain detectable for months to years following resolution of infection.	Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed. If both tests are equivocal consider repeat testing in 7–14 days if clinically warranted.
Positive/ Equivocal	Positive/ Equivocal	Positive/ Equivocal	IgM and IgG-class antibodies to <i>B. burgdorferi</i> (Lyme disease) detected.	Results are consistent with <i>B. burgdorferi</i> infection (Lyme disease) in the recent or remote past. Antibodies may remain detectable for months to years following resolution of infection.	Results should not be used to monitor or establish adequate response to therapy. Response to therapy is confirmed through resolution of clinical symptoms; additional laboratory testing should not be performed. If both tests are equivocal consider repeat testing in 7–14 days if clinically warranted.

a Performing testing outside of the indicated sequence of assays is not recommended and has not been cleared by FDA.

b Testing for IgM antibodies to *B. burgdorferi* is not indicated in patients presenting >30 days post-symptom onset.

SPECIAL CONSIDERATIONS

This section is intended to help healthcare providers better understand laboratory diagnosis for Lyme disease and any issues they may encounter.

Interpreting Results

What tests are recommended for diagnosis of Lyme disease?

CDC recommends the use of tests that have been cleared by FDA.¹⁶ Many FDA-cleared tests are available for serologic diagnosis of Lyme disease.⁸ Currently, all of these tests involve a two-step testing process. STTT consists of an FDA-cleared first tier immunoassay followed by an FDA cleared second tier immunoblot. MTTT consists of two separate immunoassays that together have been cleared by FDA for this use.

Some laboratories offer tests that have not been cleared by FDA (e.g., molecular tests, antibody tests on samples other than serum). Use of these tests is generally not recommended, as their accuracy and clinical usefulness have not been adequately established. We also caution against the use of alternative or laboratory-specific criteria for interpretation of serologic test results. Please see above tables for recommended interpretive criteria.

What if the lab only performs one of the two tiers?

Providers should order Lyme disease testing that follows the complete two-tiered testing process, whether using the standard or modified algorithm. Performing only one of the tiers may lead to incorrect results. However, in cases where the first-tier test is negative, no further testing is indicated.

If only a first-tier immunoassay is performed and the result is positive or equivocal, it should be followed by an FDA-cleared second-tier test (either an immunoblot or a second immunoassay for the MTTT algorithm).

Some laboratories may offer the option of ordering only a second-tier test (ie., only the immunoblot), however this should be ordered only if a prior first-tier immunoassay on the same sample was positive or equivocal. If only an immunoblot is performed and a first-tier test result is not available, it should be interpreted with caution (see Table 2).

Interpretation of Lyme disease serologic test results depends on knowing how long the patient has been ill. What should I do if I don't know the duration of symptoms?

In general, IgM tests should be disregarded if the patient's symptoms have lasted more than 30 days. If the patient has been sick for longer, only IgG results should be interpreted. If the patient is not sure if 30 days have passed, repeat testing may be indicated.

Labs routinely run both IgM and IgG tests, because they often do not have accurate information on the patient's duration of symptoms. Thus, it is important for providers to understand that IgM results are not useful after more than 30 days. Patients who have had Lyme disease for more than 30 days and have not been treated typically have positive IgG results.

What does it mean when repeated Lyme disease tests produce conflicting results?

Conflicting Lyme disease test results could occur for several reasons:

1. The samples being tested were taken at different times during the course of illness.
2. Different tests were used.
3. Different criteria were used to interpret the results.
4. An unrelated underlying illness caused interference with serologic testing.

Regardless of the results, it is important to verify that the test(s) used have been cleared by the FDA and are interpreted using recommended criteria as described in the preceding tables.

Are point-of-care tests for Lyme disease available?

A point-of-care test for first-tier testing using the STTT method has been cleared by the FDA. It is available as a waived test under the Clinical Laboratory Improvement Amendments (CLIA), meaning that it is a simple test with an insignificant risk of an erroneous result. Importantly, a positive or equivocal result on this test must still be followed by an approved second-tier test. Also, depending on duration of symptoms, testing may be negative by this assay in patients presenting very early following infection and repeat testing may be necessary.

Over-the-counter tests performed by patients have not been FDA-cleared and are not recommended.

Is it necessary to test samples at a laboratory that specializes in Lyme disease testing?

Serologic testing for Lyme disease is common. Thus, most general clinical laboratories perform reliable testing using FDA-cleared tests for Lyme disease or can refer samples to a laboratory that can. If a specialty laboratory is used, ensure that they are using FDA-cleared tests and recommended interpretation criteria.

Are all the bands on an immunoblot equally specific?

No. Some bands may cross-react with serum from patients who have conditions other than Lyme disease, such as syphilis, autoimmune diseases and Epstein Barr Virus (mononucleosis). In particular, the 41 kDa band is frequently cross-reactive. This is why it is important to follow the recommended criteria for interpretation of immunoblots.

General

How does previous vaccination affect Lyme disease testing?

Patients who have received the LYMERix vaccine may have positive results on whole-cell immunoassays. Providers and laboratories should consider using first-tier tests targeted to specific antigens rather than whole-cell assays for patients who have been vaccinated. The antigen contained in the LYMERix vaccine is not scored on the immunoblot, so prior vaccination should not affect immunoblot specificity. Other vaccines may be approved in the future, and it is currently unclear how these may affect serological reactivity to diagnostic tests.

How long do antibodies to Lyme disease last? Where can I get a test to make sure that a patient is cured?

Antibodies produced by the human immune system to fight off the Lyme disease bacteria (*B. burgdorferi*) can persist long after the infection is gone. This means that if a patient's blood tests positive, then it will likely continue to test positive for months or even years after successful completion of the antibiotic course, even though the bacteria are no longer present. As with many infectious diseases, there is no test that can "prove" cure.

How can I test for reinfection?

In general, serology cannot be used to differentiate a recent exposure from a past exposure. If serologic data is available from before the suspected reinfection, it may be possible to see an expanded immune response on an immunoblot. Diagnosis of reinfection relies on careful clinical consideration of exposure history and symptoms, with empiric treatment of high-risk patients.

When is it useful to repeat testing for Lyme disease?

Repeat testing may be useful if initial testing was performed too early in the window period (time period between infection and the development of antibodies that can be detected by serologic assays). In this case, it would be useful to document seroconversion. If seroconversion does not occur, it is possible that very early treatment may have

blunted the immune response or, alternatively, another condition may be responsible for the current symptoms.

Repeat testing may also be useful if initial test results are unclear. For example, if IgM and IgG results are not consistent between the first- and second-tier tests, it may be helpful to repeat testing (see Table 1b).

Repeat testing is not useful for monitoring treatment response.

When is it useful to test for antibodies to *Borrelia* species which cause Lyme disease in Europe?

In addition to the testing described in this document, some laboratories offer testing for additional species of *Borrelia* that are not found in the United States but cause Lyme disease in Europe. These tests are not appropriate for patients with no travel history outside the US.

Serologic testing for Lyme disease acquired outside of the US is recommended for patients with consistent symptoms and a history of residence in or travel to places where the disease is endemic. Outside of North America, the incidence of Lyme disease is highest in central and eastern European countries. It is considered endemic east from the British Isles into central Russia and south of from Scandinavia into the northern Mediterranean countries. Tests used to diagnose domestic (US) Lyme disease may not reliably identify internationally acquired infections.¹⁷

What Patients Should Know About Lyme Disease Testing

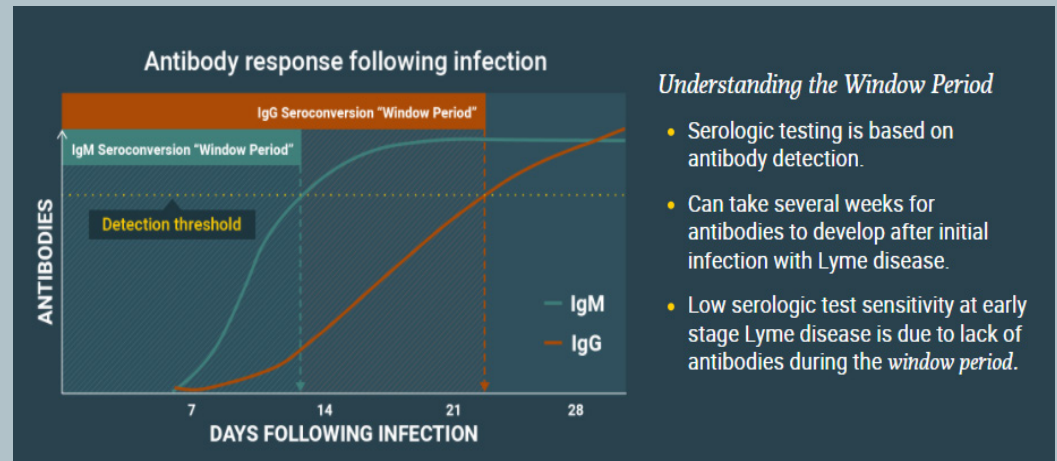
Lyme disease tests are designed to measure antibodies in your blood. Antibodies are proteins made by your body that help you fight infections.

When first infected, the body makes a type of antibody called IgM. Later, it makes a longer lasting type of antibody called IgG. Tests for IgM antibodies can detect a Lyme disease infection earlier than IgG tests. However, they are less specific and more likely to give false positive results. During the first month after illness begins, your healthcare provider should order tests for both IgM and IgG antibodies to Lyme disease. If you have been ill for a month or longer, your body will have had enough time to start making IgG antibodies. In this case, your healthcare provider should only order or interpret results from the more accurate IgG tests. Some newer Lyme disease tests can detect both types of antibodies and don't distinguish between IgM and IgG. In some cases, if you are given antibiotics promptly and your infection was quickly cured, your body may not develop enough antibodies for the Lyme disease test to become positive.

If you have had Lyme disease, you will most likely still have antibodies to the bacteria a long time after treatment. You may even test positive for years after you were ill. This does not mean that you still have the disease. However, you can have Lyme disease from a tick bite again even when you have antibodies to it.

Laboratories use a two-step process to test your blood for antibodies to Lyme disease. Using both tests together gives the best chance of correctly detecting Lyme disease infection. To ensure that the results are correct, the tests must be approved by the Food and Drug Administration (FDA) and performed in an accredited laboratory or healthcare provider's office. FDA approval means that the test has been carefully reviewed and shown to be reliable. Non-FDA approved tests may or may not work as the laboratory claims that they do. For a laboratory to be accredited, they must show inspectors that they are following standards for testing.

Lyme disease can sometimes be diagnosed and treated without laboratory testing. If you have a characteristic rash (red, gradually expanding, sometimes "bull's-eye" in appearance) and you live in or have recently traveled to an area where Lyme disease is common, testing may not be necessary to identify infection or initiate treatment.



What Providers Should Know About Lyme Disease Testing

Scenarios for which Lyme disease serologic testing is **NOT** recommended include:

- Presence of erythema migrans in high incidence areas.
- Absence of likely *Ixodes* tick exposure ([Regions Where Ticks Live](#) | [Ticks Home](#) | [CDC](#))
- Lack of travel to, or residence in a Lyme disease endemic area ([Lyme Disease Maps: Most Recent Year](#) | [Lyme Disease](#) | [CDC](#)).
- Following completion of one or more antibiotic course(s) for Lyme disease:
 - Testing should not be used to monitor response to therapy or determine ‘cure.’
- Due to pressure from patient or patient representatives in the absence of clinical criteria supporting risk for Lyme disease infection.

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