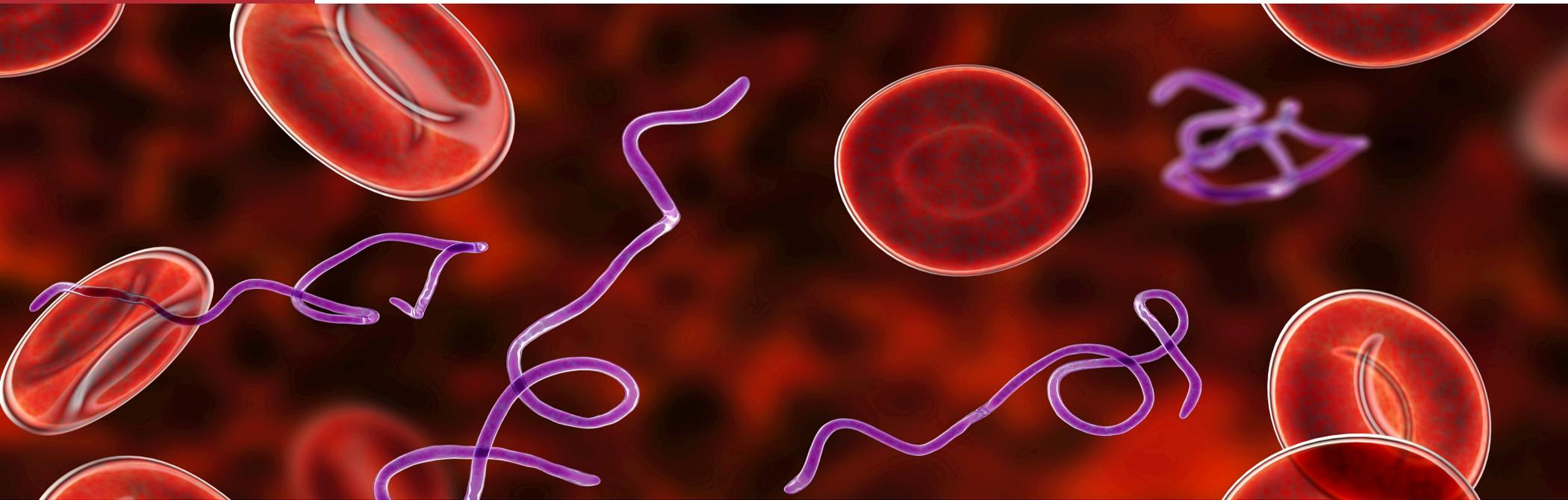# Suggested Reporting Language, Interpretation and Guidance For Lyme Disease Serologic Test Results

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APHL

ASSOCIATION OF PUBLIC HEALTH LABORATORIES®

Contents

Acknowledgments

BACKGROUND AND PURPOSE

Lyme disease, predominantly caused by the bacterial spirochete *Borrelia burgdorferi* (also referred to as *Borrelia burgdorferi*),[1] is the most common tick-borne illness in the United States. In recent years, approximately 40,000 Lyme disease cases have been reported annually to the US Centers for Disease Control and Prevention (CDC). The true incidence of this disease, however, is likely much higher as millions of diagnostic tests are performed annually.[2–6] Diagnosis of Lyme disease is reliant on both a clinical suspicion of disease and appropriate utilization of diagnostic tests, including serologic assays for detection of an immune response against *B. burgdorferi*. The recommended serologic testing algorithms involve multiple tests, and correct interpretation of results depends on the timing of testing relative to symptom onset.

Differences in language and formatting of diagnostic test reports used by clinical laboratories can lead to misinterpretation of results and confusion by both healthcare providers and patients, which may lead to misdiagnosis and poor patient health outcome. 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 of results to clinicians, public health agencies and patients.

The intent of this document is to clearly outline scenarios when Lyme disease serologic testing is indicated and to outline proper application of the standard two-tier testing (STTT) or modified two-tier testing (MTTT) algorithms for testing of human samples. These serologic approaches for the diagnosis of Lyme disease, including recommended result reporting and interpretative guidance are summarized below. Additionally, recommended standard reporting language for the STTT and MTTT are provided, with an emphasis on clear and concise interpretations to provide clarity for clinicians, laboratorians 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 testing technology.

INTRODUCTION

Lyme disease is a bacterial disease transmitted to humans through the bite of infected *Ixodes* spp. ticks. The identification of *B. burgdorferi* as the causative agent of Lyme disease in 1982[7] initiated the development of many tests by multiple in vitro diagnostic 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] In untreated infection, *B. burgdorferi* are transiently found in the bloodstream or spinal fluid, leading to general insensitivity of direct detection methods such as culture or PCR for these specimen types.[9,10] Due to this limitation, diagnostic testing for Lyme disease relies on detecting a patient's antibody response to the spirochete.

Initial diagnostic tests for Lyme disease included a variety of serologic assays, which demonstrated a lack of inter- and intra-assay precision and accuracy, necessitating standardization. Consequently, 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 evaluation of performance data across the different testing platforms, it was determined that no single serologic test for Lyme disease was sufficiently sensitive and specific to diagnose Lyme disease as a standalone assay. As a result, the standard two-tiered testing (STTT) algorithm for serologic diagnosis of Lyme disease was agreed upon to maximize clinical sensitivity and specificity. 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 Lyme disease.[12]

Rationale for Document Update

This document is updated as new or additional information becomes available and/or changes to the availability of FDA-cleared assays for the detection of antibodies to *B. burgdorferi* occur. This update to the APHL Suggested Reporting Language, Interpretation and Guidance Regarding Lyme Disease Serologic Test Results supersedes the previous version and includes information related to new FDA-cleared tests, as well as general updates and clarifications.

Clinical Description and Role of Diagnostic Testing

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.[13] 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.[14] Annular erythematous lesions occurring within several hours of a tick bite typically represent hypersensitivity reactions and are not EM. Patients who have an EM lesion, identified by a healthcare provider, and who live in or have traveled to a Lyme disease-endemic area can be diagnosed with acute Lyme disease without laboratory diagnostic support. Importantly, serologic testing of patients presenting with EM lesions is less sensitive during this acute stage of infection as compared to later stages of disease.[5] Other signs and symptoms of untreated Lyme disease may appear weeks to months after a tick bite. The organism can disseminate to the nervous system, heart, and joints. Neurologic manifestations of Lyme disease include lymphocytic meningitis, facial nerve palsy, or radiculoneuropathy. Lyme carditis, characterized by atrioventricular (AV) heart block, may also occur and if left untreated, can result in sudden death. Late Lyme disease is often characterized by arthritis, accompanied by severe joint pain and swelling, particularly in the knees and other large joints. Serologic testing is both sensitive (>87%) and specific (99%) for manifestations of disseminated Lyme disease[9] 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 infection.[15] 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 ticks. Serologic tests using FDA-cleared methods and recommended interpretive criteria should be used.[11,12] Lyme disease diagnostic tests are sensitive and specific if performed as recommended at appropriate times post-infection or for manifestations of disseminated disease. False positive tests 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] Conversely, 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.

STTT: SUGGESTED RESULT REPORTING AND INTERPRETATION

STTT begins with an immunoassay detecting IgM and/or IgG antibodies to *B. burgdorferi*. A variety of different immunoassay formats have been FDA-cleared for first tier testing (e.g., enzyme, fluorescent, chemiluminescent, lateral flow, etc.). If the initial first tier 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 interpreted to guide clinical decisions. For samples collected over 30 days post symptom onset, only the anti-*B. burgdorferi* IgG immunoblot should be considered. For detailed information regarding result interpretation, please see **Table 1a** (Total Ig Immunoassay as First Tier Assay) or **Table 1b** (Separate IgM and IgG immunoassays as First Tier Assays).

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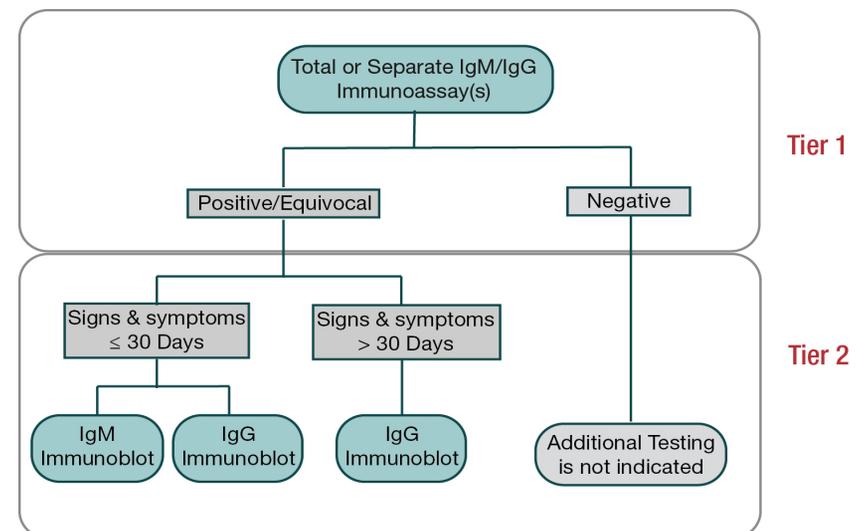
Table 1a. Suggested Guidance for Reporting Results from the Standard Two-Tiered Lyme Disease Serologic Testing Using a Total Ig Immunoassay as a First Tier Assay

Test Sequence			Interpretation for Laboratories	Interpretation for Providers	Comments/Further Actions (may be included on the laboratory report)
Tier 1: Total Ig Immunoassay	Tier 2a: IgM Immunoblot[a,b]	Tier 2b: IgG Immunoblot[c]			
Negative	Testing Not Indicated[d]	Testing Not Indicated[d]	Negative for antibodies to *B. burgdorferi* (Lyme disease).	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7-14 days is recommended.
Positive/Equivocal	Negative	Negative[e]	Antibodies to *B. burgdorferi* (Lyme disease) not confirmed.	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7-14 days is recommended.
Positive/Equivocal	Positive[e]	Negative[e]	IgM-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with acute or recent infection with *B. burgdorferi* (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.
Positive/Equivocal	Negative	Positive[e]	IgG-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with *B. burgdorferi* infection (Lyme disease) 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.
Positive/Equivocal	Positive[e]	Positive[e]	IgM- and IgG-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with *B. burgdorferi* 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.

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 Laboratories may choose to report individual bands when the overall test is positive and individual IgG bands when the overall test result is negative. Reporting of individual IgM bands when the overall test is negative is not recommended.

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

Test Sequence			Interpretation for Laboratories	Interpretation for Providers	Comments/Further Actions (may be included on the laboratory report)
Tier 1: Separate IgM and IgG Immunoassays	Tier 2a: IgM Immunoblot[a,b]	Tier 2b: IgG Immunoblot[c]			
Concordant *Tier 1 IgM and IgG Immunoassay results*					
Negative	Testing Not Indicated[d]	Testing Not Indicated[d]	Negative for antibodies to *B. burgdorferi* (Lyme disease).	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7-14 days is recommended.
Positive/Equivocal by both assays	Negative	Negative[e]	Antibodies to *B. burgdorferi* (Lyme disease) not confirmed.	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7-14 days is recommended.
Positive/Equivocal by both assays	Positive[e]	Negative[e]	IgM-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with acute or recent infection with *B. burgdorferi* (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.
Positive/Equivocal by both assays	Negative	Positive[e]	IgG-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with *B. burgdorferi* infection (Lyme disease) 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.
Positive/Equivocal by both assays	Positive[e]	Positive[e]	IgM- and IgG-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with *B. burgdorferi* 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.

Test Sequence			Interpretation for Laboratories	Interpretation for Providers	Comments/Further Actions (may be included on the laboratory report)
Tier 1: Separate IgM and IgG Immunoassays	Tier 2a: IgM Immunoblot[a,b]	Tier 2b: IgG Immunoblot[c]			
Discordant *Tier 1 IgM and IgG Immunoassay results*					
IgM Positive/ Equivocal IgG Negative	Negative	Not indicated or if performed, results should not be considered for clinical care.	Negative for antibodies to *B. burgdorferi* (Lyme disease).	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7–14 days is recommended.
IgM Positive/ Equivocal IgG Negative	Positive[e]	Not indicated or if performed, results should not be considered for clinical care.	IgM-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with acute or recent infection with *B. burgdorferi* (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 Negative/Not performed IgG Positive/Equivocal	Not indicated or if performed, results should not be considered for clinical care.	Negative[d]	Negative for antibodies to *B. burgdorferi* (Lyme disease).	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7-14 days is recommended.
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 *B. burgdorferi* (Lyme disease) detected.	Results are consistent with *B. burgdorferi* infection (Lyme disease) 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.
Discordant *Tier 1 & 2 IgM and IgG Immunoassay results*					
IgM Positive/ Equivocal IgG Negative	Negative	Positive[d]	Inconclusive	Repeat testing using a new sample. If results remain inconclusive, consider testing using a different algorithm.	Consider further testing or alternate diagnosis.
IgM Negative/Not performed IgG Positive/ Equivocal	Positive[d]	Negative[d]	Inconclusive	Repeat testing using a new sample. If results remain inconclusive, consider testing using a different algorithm.	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. Laboratories may choose to report individual bands when the overall test is positive, and individual IgG bands when the overall test result is negative. Reporting of individual IgM bands when the overall test is negative is not recommended.

STTT: RESULTS OF FIRST TIER TESTING UNKNOWN

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 increased probability 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. These immunoblot-only test options are intended to be ordered by laboratories that only offer first-tier *B. burgdorferi* testing at their site. Reference laboratories that offer *B. burgdorferi* immunoblot-only orderable test codes assume that samples submitted for this test have been screened positive or equivocal by a first-tier *B. burgdorferi* immunoassay. See **Table 2** for suggested guidance for interpreting results under these circumstances.

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Table 2. Suggested Guidance For Reporting Tier 2 Results in the STTT When Results of Tier 1 Results are Unknown or Assumed Positive/Equivocal

Test Sequence			Algorithm Interpretations	Interpretation for Laboratory Report	Comments/Further Actions (may be included on the laboratory report)
Tier 1: Total Ig Immunoassay	Tier 2a: IgM Immunoblot[a,b]	Tier 2b: IgG Immunoblot[c]			
Unknown or Assumed Positive/Equivocal	Negative	Negative	Antibodies to *B. burgdorferi* (Lyme disease) not confirmed.	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with B. burgdorferi. If recent infection is suspected, repeat testing of a new sample collected in 7-14 days is recommended using the two-tiered testing algorithm.
Unknown or Assumed Positive/Equivocal	Positive[d]	Negative	IgM-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Interpretation of these results is only accurate if an initial *B. burgdorferi* immunoassay was positive or equivocal. If so, results are consistent with acute or recent infection with *B. burgdorferi* (Lyme disease).	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.
Unknown or Assumed Positive/Equivocal	Negative	Positive	IgG-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Interpretation of these results is only accurate if an initial *B. burgdorferi* immunoassay was positive or equivocal. Results are consistent with *B. burgdorferi* infection (Lyme disease) 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.
Unknown or Assumed Positive/Equivocal	Positive[d]	Positive	IgM- and IgG-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Interpretation of these results is only accurate if an initial *B. burgdorferi* immunoassay was positive or equivocal. Results are consistent with *B. burgdorferi* 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.

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. burgdorferii*. 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 Laboratories may choose to report individual bands when the overall test is positive, and individual IgG bands when the overall test result is negative. Reporting of individual IgM bands when the overall test is negative is not recommended.

MTTT: SUGGESTED RESULT REPORTING AND INTERPRETATION

The MTTT algorithm differs from the STTT algorithm in that the second-tier assay(s) are immunoassays, not immunoblots. Importantly, the immunoassays used as part of an MTTT algorithm must have received FDA clearance for the combination of assays and the tier in which they are used. MTTT assays are based on multiple, different *B. burgdorferi* antigens; assays used in pairs necessarily detect different antigens. MTTT algorithms begin 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 for detection of either total IgM/IgG (**Figure 2** and **Table 3**) or separated IgM and IgG (**Figure 3** and **Table 4**). Although MTTT is recommended to be performed in sequence, some approved platforms can perform both tests concurrently. If such a concurrent testing approach is implemented, results of the second-tier assays should only be reported if first tier results are positive/equivocal.

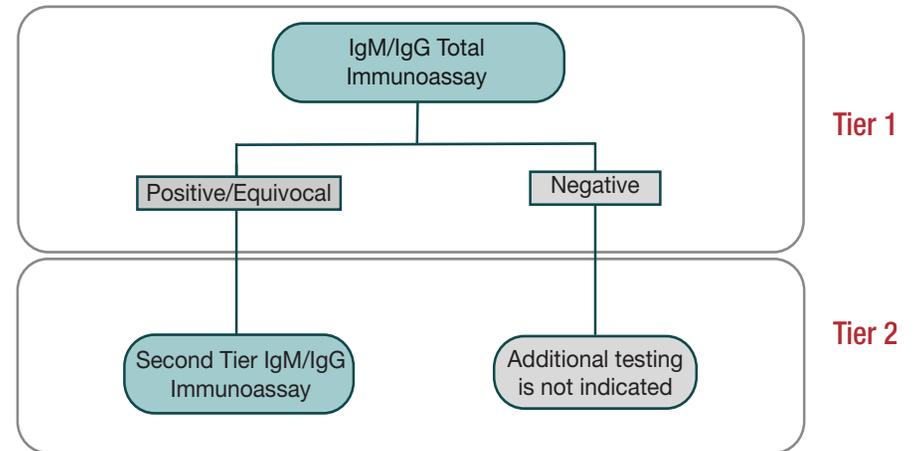Figure 2. MTTT Algorithm 1 - Two Total IgM/IgG Immunoassays

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: IgM/IgG Total Immunoassay	Tier 2: IgM/IgG Total Immunoassay			
Negative	Not Indicated or If performed, results should not be considered for clinical care.	Negative for antibodies to *B. burgdorferi* (Lyme disease).	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7–14 days is recommended.
Positive/ Equivocal	Negative	Antibodies to *B. burgdorferi* (Lyme disease) not confirmed.	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7–14 days may be considered to confirm infection.
Positive/ Equivocal	Positive/Equivocal[b]	IgM- and/or IgG-class antibodies to *B. burgdorferi* (Lyme disease) detected. Specific antibody class detected cannot be determined.	Results are consistent with *B. burgdorferi* 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 tiers are equivocal consider repeat testing in 7–14 days if clinically warranted.

a Testing must be performed using assays that have been FDA-cleared together for this purpose.

b Equivocal results from the Tier 2 Immunoassay should be reported as positive per the package insert and interpreted as supportive evidence for the presence of IgM/IgG antibodies and exposure to B. burgdorferi.

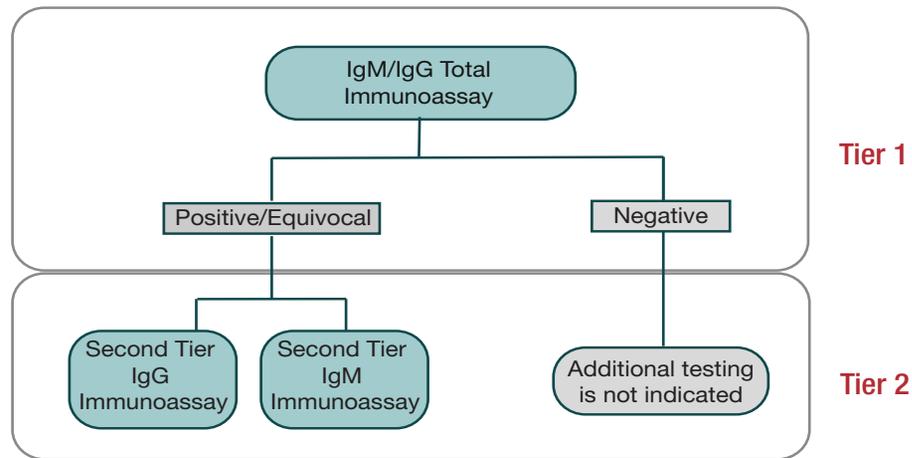Figure 3: MTTT Algorithm 2 – Separate IgM and IgG Second Tier Immunoassays

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: IgM/ IgG Total Immunoassay	Tier 2a: IgM Immunoassay	Tier 2b: IgG Immunoassay			
Negative	Not Indicated or If performed, results should not be considered for clinical care.	Testing Not Indicated/Negative	Negative for antibodies to *B. burgdorferi* (Lyme disease).	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7–14 days is recommended.
Positive/Equivocal	Negative	Negative	Antibodies to *B. burgdorferi* (Lyme disease) not confirmed.	No laboratory evidence of infection with *B. burgdorferi* (Lyme disease).	Negative results may occur in patients recently infected (≤14 days) with *B. burgdorferi*. If recent infection is suspected, repeat testing on a new sample collected in 7–14 days to demonstrate seroconversion may be considered to confirm infection.
Positive/Equivocal	Positive/ Equivocal[b]	Negative	IgM-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with acute or recent infection with *B. burgdorferi* (Lyme disease).	In untreated patients who have been sick for more than 30 days, positive IgM results should be interpreted with caution if IgG results are negative. Consider testing a new specimen collected in 7–14 days to demonstrate seroconversion.

Table 4 (cont'd)

Test Sequence			Interpretation for Laboratories	Interpretation for Providers	Comments/Further Actions (may be included on the laboratory report)
Tier 1: IgM/ IgG Total Immunoassay	Tier 2a: IgM Immunoassay	Tier 2b: IgG Immunoassay			
Positive/Equivocal	Negative	Positive/Equivocal[b]	IgG-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with *B. burgdorferi* infection (Lyme disease) 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.
Positive/Equivocal	Positive/Equivocal[b]	Positive/Equivocal[b]	IgM and IgG-class antibodies to *B. burgdorferi* (Lyme disease) detected.	Results are consistent with *B. burgdorferi* 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.

a. Testing must be performed using assays that have been FDA-cleared together for this purpose.

b. Equivocal results from the Tier 2 Immunoassay should be reported as positive per the package insert and interpreted as supportive evidence for the presence of IgM and/or IgG antibodies and exposure to B. burgdorferi

FREQUENTLY ASKED QUESTIONS

This section is intended to help healthcare providers or public health personnel 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.[9,10] Currently, all recommended algorithms 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. Use of these tests is not recommended, as their accuracy and clinical usefulness have not been adequately established. Additionally, use of alternative or laboratory-specific criteria for interpretation of serologic test results is not recommended. 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 recommended two-tiered testing process, whether using the standard or modified algorithm. Performing only one of the tiers may lead to incorrect interpretation. 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 immunoblot for the STTT algorithm. As MTTT tests are implemented as pairs, it is unlikely that a laboratory would perform only one of the FDA-cleared assays in an algorithm.

Some laboratories may offer the option of ordering only a second-tier test (i.e., immunoblot), however this should be ordered on the same sample only if a prior first-tier immunoassay 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?

STTT: Many laboratories only offer IgM and IgG immunoblot testing concurrently. However, in the STTT algorithm, 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. For patients who are unsure of their symptom duration and initial serologic testing is negative, repeat testing on a new sample collected in 7-14 days may be beneficial.

MTTT: The 30-day restriction on interpretation of IgM test results is not specifically indicated in the manufacturer instructions for use for assays cleared as part of a MTTT algorithm. However, providers should be aware that patients who have had Lyme disease for more than 30 days and have not been treated are typically positive for IgG antibodies. For untreated patients who have been sick for more than 30 days, positive IgM results should be interpreted with caution if IgG results are negative.

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.

How should I interpret equivocal results on an MTTT?

Some manufacturers of tests used in MTTT algorithms have formatted their tests so they can produce equivocal results in addition to positive and negative results. For the purposes of interpretation, an equivocal result is considered comparable to a positive result. Thus, equivocal results on both steps are interpreted as an overall positive result. Similarly, an equivocal result on either step, combined with a positive result on the other step, indicates an overall positive result. FDA evaluated all performance characteristics prior to approval, taking these situations into account.

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 mononucleosis (Epstein Barr Virus). 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

Are point-of-care or at-home tests for Lyme disease available?

A point-of-care test that functions as a first-tier test 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 and must be reported to the health department per local reporting regulations, similar to any positive test for Lyme disease performed by in a laboratory. 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 sample collection kits have not been FDA-cleared and are not recommended.

How does previous vaccination affect Lyme disease testing?

Patients who received the LYMERix vaccine, which was discontinued in 2002, may still 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 were previously 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 and provision of treatment relies on careful clinical consideration of exposure history and symptoms.

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 which cause Lyme disease in Europe. These tests are not appropriate for patients without a travel history outside the US.

Serologic testing for Lyme disease acquired outside of the United States 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 from Scandinavia into the northern Mediterranean countries. The immunoblots used as part of the STTT algorithm in North America are specific to *B. burgdorferi* and are unlikely to detect infection with other species causing Lyme disease.[17,18] However, MTTT assays are likely to detect antibodies to a wider range of Lyme disease-causing Borrelia species.

Two positive EIAs from the same specimen were reported to our health department. How can I tell if these are FDA-cleared MTTT assays?

When MTTT algorithms were first implemented by commercial laboratories, there were no specific LOINC codes to distinguish these assays from any other first-tier assay used in STTT algorithms, making it challenging for health departments to identify MTTT. However, specific LOINC codes for MTTT assays were issued in early 2023. It remains incumbent upon the performing laboratory to choose the most accurate LOINC codes for their tests. Direct contact with reporting laboratories in each public health jurisdiction is recommended to understand test menus and associated LOINC codes and to ensure capture of all relevant positive laboratory tests for public health surveillance purposes. The most recent information on currently available MTTT and STTT assays and the recommended mapping are available on the CDC's LOINC In Vitro Diagnostic (LIVD) Test Code Mapping Website.

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. If you have been ill for a month or longer, your body will have had enough time to start making IgG antibodies. 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.

Antibody response following infection

Understanding the Window Period

- Serologic testing is based on antibody detection.
- Can take several weeks for antibodies to develop after initial infection with Lyme disease.
- Low serologic test sensitivity at early stage Lyme disease is due to lack of antibodies during the *window period*.

If you have had Lyme disease, you will most likely still have antibodies to the bacteria for 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 get Lyme disease again even when you have antibodies from a prior infection.

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 US 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 "bullseye" 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.'
- Pressure from patient or patient representatives in the absence of clinical criteria supporting risk for Lyme disease infection.
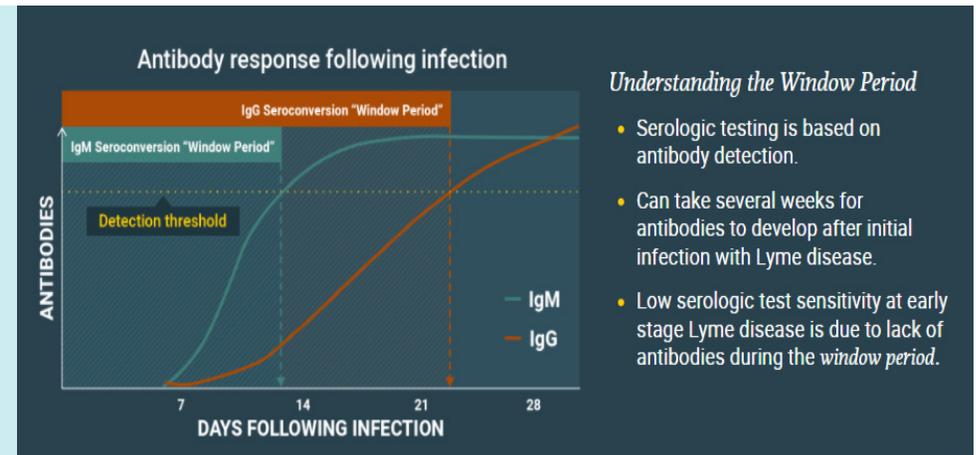
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