avalon

CareSource

Qualified Health Plans offered in North Carolina by CareSource North Carolina Co., d/b/a CareSource

Lyme Disease Testing

Policy Number: AHS – G2143 – Lyme Disease Testing	Effective Date: 09/01/2025

I. Policy Description

Lyme disease is a common multisystem inflammatory disease caused by spirochetes of the family *Borreliaceae* transmitted through the bite of an infected tick of the genus *Ixodes.*[1] Lyme disease affects the skin in its early localized stage, and spreads to the joints, nervous system, and other organ systems in its later disseminated stages.[2]

II. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request.

1) For individuals with symptoms of Lyme disease and a history of travel to a region endemic for Lyme (with or without a history of a tick bite), serologic testing (2-tier testing strategy using a sensitive enzyme immunoassay (EIA) or immunofluorescence assay, followed by a western immunoblot assay or FDA-cleared second EIA assay) **MEETS COVERAGE CRITERIA**.

2) For individuals with a history of travel to a region endemic for Lyme, serologic testing (2-tier testing strategy using a sensitive enzyme immunoassay (EIA) or immunofluorescence assay, followed by a western immunoblot assay or FDA-cleared second EIA assay) **MEETS COVERAGE CRITERIA** in any of the following situations:

   a) For individuals with acute myocarditis/pericarditis of unknown cause.

   b) For individuals with meningitis, encephalitis, or myelitis.

   c) For individuals with painful radiculoneuritis.

   d) For individuals with mononeuropathy multiplex including confluent mononeuropathy multiplex.

   e) For individuals with acute cranial neuropathy.

3) Serologic testing **DOES NOT MEET COVERAGE CRITERIA** in any of the following situations:

a) For individuals with an erythema migrans (EM) rash (patients with skin rashes consistent with EM who reside in or who have recently traveled to an endemic area should be treated for Lyme disease).

b) To screen asymptomatic patients living in endemic areas.

c) For individuals with non-specific symptoms only (e.g., fatigue, myalgias/arthralgias).

d) For individuals with amyotrophic lateral sclerosis.

e) For individuals with relapsing-remitting multiple sclerosis.

f) For individuals with Parkinson's disease.

g) For individuals with dementia or cognitive decline, or new-onset seizures.

h) For individuals with psychiatric illness.

4) Detection of *Borrelia burgdorferi* by nucleic acid identification techniques (direct or amplified probe) **DOES NOT MEET COVERAGE CRITERIA**.

5) For individuals who have previously tested positive for Lyme disease, repeat serologic testing **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

6) All other testing for *Borrelia burgdorferi* not described above **DOES NOT MEET COVERAGE CRITERIA**.

7) For the diagnosis of Lyme disease, testing of the individual tick **DOES NOT MEET COVERAGE CRITERIA.**

III. Table of Terminology

Term	Definition
AAN	The American Academy of Neurology
AAP	American Academy of Pediatrics
ACR	The American College of Rheumatology
ACEIA	Antibody-capture enzyme immunoassay
CCDR	Canada Communicable Disease Report
CD57	Cluster designation 57
CDC	Centers for Disease Control and Prevention
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CNS	Central nervous system

CPS	Canadian Paediatric Society
CSF	Cerebrospinal fluid
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
EM	Erythema migrans
FDA	Food and Drug Administration
HDPCR	High-definition polymerase chain reaction
IDEG	Infectious Disease Expert Group
IDSA	The Infectious Diseases Society of America
IFA	Immunofluorescence assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LD	Lyme disease
LDT	Laboratory developed test
LNB	Lyme neuroborreliosis
MTTT	Modified two-tiered testing
NICE	National Institute for Health and Care Excellence
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PHAC	Public Health Agency of Canada
PNS	Peripheral nervous system
POC	Point of care
PPV	Positive predictive value
PTLDS	Post-Lyme disease syndrome
RUO	Research use only
STTT	Standardized two-tier testing
TBP	Tick-borne pathogen
WB-RTPCR	Whole blood real-time polymerase chain reaction
xVFA	Multiplexed vertical flow assay

IV. Scientific Background

Lyme disease can be caused by several species in the spirochete family *Borreliaceae;* however, infection in North America is predominately caused by *B. burgdorferi.* Much less commonly, in the upper midwestern United States, cases have been associated with *B. mayonii.*[3,4] The taxonomic classification system for this species is undergoing revision, and the genus name may be represented as either *Borrelia* or *Borreliella.*[5,6] *Borrelia burgdorferi* occurs naturally in reservoir hosts, including small mammals and birds.[7] *Ixodes scapularis* and *I. pacificus* become infected with *B. burgdorferi* while feeding on the blood of natural reservoir hosts. Transmission to humans results from the bite of an infected tick.[8] Spirochete transmission times and virulence

depend upon the tick and *Borrelia* species, and infection can never be excluded after a tick bite irrespective of the estimated duration of attachment time.[9]

In the earliest stage of Lyme disease, *B. burgdorferi* disseminates from the site of the tick bite resulting in the colonization of dermal tissue and localized infection characterized by a painless bulls-eye rash called erythema migrans (EM), experienced by approximately 70–80% of patients at the site of the tick bite. This is accompanied by non-specific flu-like symptoms, including headache, neck stiffness, malaise, fatigue, myalgia, and fever. During localized infection, the number of *B. burgdorferi* cells increases in the dermal tissue. If left untreated, *B. burgdorferi* can disseminate from the site of the tick bite through the bloodstream and/or lymphatic system to invade and colonize various tissues days to weeks after infection. This can affect the heart, joints, and nervous system. Months to years after exposure to *B. burgdorferi,* affected individuals can experience different manifestations, including neuroborreliosis, Lyme carditis, and arthritis.[7]

Over 63,000 cases of Lyme disease were reported to the CDC by state health departments and the District of Columbia in 2022. The CDC reports that about 476,000 Americans are diagnosed and treated for Lyme disease each year, however this estimate likely includes patients who are treated based on clinical suspicion but do not actually Lyme disease.[10]

Even following antibiotic treatment, a subset of patients continue to present with arthritic symptoms; this has been designated as postinfectious, antibiotic-refractory Lyme arthritis.[7] The term "post-Lyme disease syndrome" (PTLDS) is often used to describe the nonspecific symptoms (such as headache, fatigue, and arthralgias) that may persist for months after treatment of Lyme disease. For the majority of patients, these symptoms improve gradually over six months to one year.[2] Weitzner, et al. (2015) found that "PTLDS may persist for over 10 years in some patients with culture-confirmed early Lyme disease. Such long-standing symptoms were not associated with functional impairment or a particular strain of *B. burgdorferi*."

The diagnosis of Lyme disease is based on an individual's history of possible exposure to ticks, the presence of characteristic signs and symptoms, and blood test results.[2] Direct detection of *Borrelia burgdorferi* has limited applications.[12] Thus, most laboratory confirmation of Lyme disease involves the detection of antibody responses against *B. burgdorferi* in serum.[13] Serology testing is not recommended for patients who do not have symptoms typical of Lyme disease[12], as current assays do not distinguish between active and past infection, thus a positive result is more likely to be a false positive. Early diagnosis of erythema migrants should be made without testing because the lesion appears prior to development of a diagnostic, adaptive immune response.[2]

Serological testing using the two-tier algorithm, comprising a first screening enzymatic immunoassay (EIA), followed by a confirmatory western blot test, is the gold standard for Lyme disease diagnoses.[2,14,15] The CDC currently recommends a two-step testing process for Lyme disease serologic testing.[16] Although STTT detection of early localized infection is poor, STTT detection of late disease is excellent.[13] Evidence of seronegative late Lyme disease is unconvincing.[17] A systematic review has shown that the sensitivity of serology for Lyme disease

in early localized infection is 50%, but the algorithm performs well in late stages of the infection, where the sensitivity approaches 100%.[18]

On July 29, 2019, the FDA approved several Lyme disease serologic assays, including ZEUS ELISA, allowing for an EIA rather than western blot as the second test in the two-tier algorithm.[19] ZEUS ELISA is a Modified Two-Tiered Testing (MTTT) Algorithm that replaces the second-tier western blot with a more sensitive and specific methodology, such as ELISA. According to ZEUS Scientific, MTTT reduces the number of missed clinically positive patient samples and improves lab efficiency.[20] Compared to the traditional STTT, the MTTT algorithms improve sensitivity to detect early infections and have equivalent sensitivity for detecting late-stage infections and comparable specificity. In addition, MTTT may have the benefit of improved sensitivity in identifying positive cases in patients infected with related strains of *Borrelia*. In a study by Davis, one case of infection with a European genospecies of *Borrelia* was detected by MTTT, which was missed by STTT.[21] The Canada Communicable Disease Report (CCDR) agrees with the FDA recommendation, advising that "Diagnostic improvements in sensitivity of [Lyme disease] testing without significant loss of specificity have been consistently reported when MTTT is compared with STTT in studies conducted in highly [Lyme disease] endemic regions."[22]

Polymerase chain reaction (PCR) testing may be useful in the early stages of a Lyme disease infection before an immune response occurs and is also helpful when testing for reinfection. Other potential techniques for Lyme disease diagnostics include cell culture, ELISA, urine testing, and multiplex testing techniques.[15]

Proprietary Testing

Other diagnostic tests have been created but not widely validated.[2] For instance, Wormser, et al. (2013) evaluated a C6 enzyme-linked immunosorbent assay (ELISA) as a single-step, serodiagnostic test that uses a reference standard of two-tier testing. This test provided increased sensitivity in early Lyme disease with comparable sensitivity in later manifestations of the disease. Four hundred and three samples were compared to the sensitivities of the traditional two-tier tests, and the C6 ELISA was measured to have a 66.5% sensitivity and a 35.2% sensitivity, both of which were more sensitive than the individual steps of the STTT approach. The specificity was evaluated with over 2200 blood donors, and the C6 ELISA was evaluated at 98.9% specificity.[23]

Urine testing for diagnosis of Lyme disease is available from multiple laboratories. For example, Igenex (2017) claims that the urine tests "are useful during the acute phase of infection before antibodies are present, in seronegative patients, in patients with vague symptoms of long duration, and previously-treated patients with recurring symptoms." However, the American Academy of Pediatrics (AAP) asserts that "a number of tests for Lyme disease have been found to be invalid on the basis of independent testing or to be too nonspecific to exclude false-positive results", including "urine tests for B burgdorferi, CD57 assay, novel culture techniques, and antibody panels that differ from those recommended as part of standardized 2-tier testing."[25]

IGeneX's proprietary immunoblot has been used to detect IgM and IgG antibodies to diagnose Lyme disease. From the sample report, IGeneX has stated that "Recombinant *B. burgdorferi* species antigens are sprayed at specific positions onto a nitrocellulose membrane and cut into strips. These strips are used to detect *B. burgdorferi* specific antibodies in patient serum."[24] Eight total species of *Borrelia* are detected by this test; based on 174 samples, the immunoblot was found to have a sensitivity of 90.9% and specificities of 98% (IgM) and 98.7% (IgG).[24] Igenex also has a PCR-based test for the detection of *B. burgdorferi*. Four hundred and two positive samples for *B. burgdorferi* were evaluated based on Igenex's proprietary PCR test and the CDC diagnostic criteria (the traditional two-tiered test). Out of the 402 samples, 236 were considered positive by the proprietary PCR test and 70 were considered positive per the CDC criteria.[26]

Researchers have introduced point-of-care (POC) serological tests for Lyme disease that uses synthetic peptides and a paper-based platform to detect LD antibodies in blood samples. The test combines multiple peptides with a machine learning model to achieve high accuracy, with 95.5% sensitivity and 100% specificity, as validated in blinded tests and CDC samples. It matches the performance of the current two-tier lab testing but is simpler and faster, offering a practical solution for earlier diagnosis, improved treatment, and immune monitoring in diverse healthcare settings.[27] However, it's important to note that the CDC still only recommends the two-step serologic testing process as the standard diagnostic method for Lyme disease.[16] The CDC states "new tests may be developed as alternatives to one or both steps of the two-step process. Before CDC will recommend new tests, they must be cleared by the FDA."[16]

Clinical Utility and Validity

Waddell, et al. (2016) assessed the accuracy of the traditional diagnostic tests of Lyme disease. A total of 11 studies with 34 lines of data were evaluated for the overall accuracy. The overall sensitivity was found to be 82%, and the overall specificity was found to be 94.2%. Fifteen studies were examined for stage one of Lyme disease, and the sensitivity was found to be 54%; however, the specificity was calculated to be 96.8%. Stage two (five studies, six lines) had a sensitivity of 79.1% and specificity of 97.7%, and stage three (nine studies, 20 lines) had a sensitivity of 94.7% and specificity of 96.1%. The CDC immunoblots (second tier, two studies, four lines) were estimated at 91% sensitivity and 99% specificity.[18]

Joung, et al. (2019) note that while the CDC recommends serological methods for Lyme disease testing, it is expensive (over $400/test) and can take longer than 24 hours to obtain results; therefore, a cost-effective and rapid assay was developed to address these challenges. This assay can detect early stage Lyme disease and "assays for antibodies specific to seven *Borrelia* antigens and a synthetic peptide in a paper-based multiplexed vertical flow assay (xVFA)"; the specificity of this test was identified at 87% and sensitivity at 90.5%.[28]

Shakir, et al. (2019) used a total of 379 whole blood samples to evaluate ChromaCode's Research Use Only (RUO) nine target High-Definition PCR (HDPCR™) Tick-Borne Pathogen (TBP) panel. Results were compared to clinically validated real-time PCR assays and laboratory developed tests. The final positive percent agreement and negative percent agreement "for the TBP panel was 97.7% (95% CI 95.2% - 99.0%) and 99.6% (95% CI 99.3% - 99.8%),

respectively, with an overall agreement of 99.5% (95% CI 99.2% -99.7%)" with the laboratory developed tests."[29]

Nigrovic, et al. (2019) evaluated the Lyme disease PCR test compared to the traditional two-tier assessment method (a positive or equivocal EIA and a positive immunoblot test). In total, 124 were tested and 54 had Lyme disease. However, only 23 of the Lyme disease patients had a positive PCR test, giving a sensitivity of 41.8% and specificity of 100%.[30] These results show that the Lyme disease PCR test has low sensitivity.

Davis, et al. (2020) evaluated the effectiveness of the MTTT algorithm compared to the STTT algorithm. MTTT algorithm uses a second enzyme immunoassay (EIA) instead of the immunoblots for samples that test positive or equivocal on the first EIA. Retrospective chart reviews were performed on 10,253 specimens tested for Lyme disease (LD) serology. "Patients were classified as having Lyme disease if they had a positive STTT result, a negative STTT result but symptoms consistent with Lyme disease, or evidence of seroconversion on paired specimens."[21] Of the 10,253 specimens, 9,806 (95.6%) were negative for LD and 447 patients tested positive. Of the 447 patients, 227 were classified as patients with LD. "Of the 227 patients classified as having LD, 65 (28.6%) had early localized infections, 67 (29.5%) had early disseminated infections, 26 (11.5%) had late LD, 61 (26.9%) had evidence of old infections, and 8 (3.5%) had posttreatment LD syndrome. Of the remaining 63 patients with early localized disease, 16 (25.4%) were positive by MTTT but negative by STTT. The MTTT identified an additional four (6.6%) cases of early disseminated infection and one case (3.8%) in late LD."[21] Overall, MTTT identified additional cases in early localized and early disseminated infections and detected 25% more early infections with a specificity of 99.56% (99.41 to 99.68%) compared to the STTT.[21]

van Gorkom, et al. (2020) evaluated the utility of an in-house and a commercial enzyme-linked immunosorbent spot (ELISpot) assay for the diagnosis of Lyme neuroborreliosis (LNB). Peripheral blood mononuclear cells (PBMCs) were isolated from eighty-seven patients diagnosed with LNB at Diakonessenhuis Hospital, Utrecht, and the St Antonius Hospital, Nieuwegein, the Netherlands between March 2014 and November 2017. In-house *Borrelia* ELISpot assay and the commercial LymeSpot assay. However, it was found that both tests performed unsatisfactorily—the sensitivity for the *Borrelia* ELISpot yielded a sensitivity of 61.1% (95% CI: 38.9-77.8%) and a specificity of 66.7(42.0-81.2%), while the LymeSpot assay produced 66.7% (95% CI: 44.4-88.9%) and 59.4% (95% 44.9-72.5%), respectively. Moreover, low PPVs for ELISpot and LymeSpot were observed (30.6% vs. 29.7%, respectively), further corroborate their poor diagnostic performance. The researchers do acknowledge a few shortcomings in their study, namely that the isolation procedure for the PBMC deviated from that of the LymeSpot assay—however, the deviations from protocol were allowed for the technician to minimize differences when comparing across assays to allow for fairer comparison of results. Though this was the case, they believe still that the deviations "from the recommended protocol are not critical", and as such they uphold "the conclusion stands that both ELISpot assays cannot help to diagnose active LNB."[31]

Sabin, et al. (2023) compared the MTTT algorithm to the STTT. The authors compared samples from 320 patients. "The MTTT confirmed the illness in 116 subjects (36%, P = 0.007), and 30 (26%) were negative by the STTT." MTTT sensitivity was increased in early infection, but insufficiently sensitive to non-*Borrelia* species infections. The authors concluded that "Routine adoption of MTTT would improve sensitivity for early Lyme disease attributable to B. burgdorferi, but may not capture illness attributed to B. mayonii and B. miyamotoi."[32]

Pratt, et al. (2022) believed that the concurrent use of molecular and serologic testing could broaden the diagnostic window for early LD. Of the 33199 specimens submitted for review by antibody-capture EIA and WB-RTPCR, 1379 tested positive, and of those positive, "1,179 were positive by serology only, 131 were positive by molecular testing only, and 69 were positive by both serology and molecular testing." Overall, they found that "4.2% of all specimens were positive and nearly 10% were detected by WB-RTPCR alone." The authors reported that "Of the 131 specimens that tested positive for *B burgdorferi* DNA only, 29 had follow-up samples submitted for follow-up serology testing". Most importantly, "Eighty-six percent (25/29) of the patients with follow-up testing demonstrated seroconversion, 3% (1/29) were equivocal, and 10% (3/29) tested negative."[33] The researchers also examined "2526 specimens submitted for concurrent MTTT and molecular testing" and found that "The two data sets showed a similar percentage of molecular-positive, serology-negative results (8.7% for MTTT and 9.5% for ACEIA)". Moreover, using the χ^2 test, they found "no statistically significant difference between the antibody-capture and MTTT data sets was observed when analyzing the Lyme-positive results" ($\chi^2 = 0.2765$, $P = .871$). Consequently, it was concluded that "WB-RTPCR, in clinically suspected cases of ELD, can identify *B burgdorferi* infection that serology testing could otherwise miss". Though a retrospective review of paired samples was used to confirm their results, the lack of clinical information to associate with the results motivates the need for a future prospective study.[33]

Arumugam, et al. (2019) developed a new multiplexed test, mChip-Ld, as a potential alternative to the standard two-tiered (STT) method for diagnosing LD. They tested the assay using 241 serum samples from patients in various stages of LD, including early, convalescent, Lyme arthritis, and post-treatment stages. The authors selected three key antigens—VlsE, a synthetic 33-mer peptide (PepVF), and OspC—to improve the test's sensitivity across all stages. With a specificity of 95%, the mChip-Ld demonstrated sensitivity ranging from 80-85% for early LD and 100% for Lyme arthritis, outperforming the STT algorithm, which had sensitivities of 48.5% to 75% for early LD. The mChip-Ld also showed high specificity (97.5% to 100%). These results suggest that the mChip-Ld could be a more sensitive, rapid, and practical POC for diagnosing LD at different stages.[34]

V. Guidelines and Recommendations

Centers for Disease Control and Prevention (CDC)

The CDC currently recommends a two-step process when testing blood for evidence of antibodies against the LD bacteria. Both steps can be done using the same blood sample.

- **The first step** uses a testing procedure called "EIA" (enzyme immunoassay) or rarely, an "IFA" (indirect immunofluorescence assay).
- **If this first step is negative**, no further testing of the specimen is recommended.
- **If the first step is positive** or indeterminate (sometimes called "equivocal"), the second step should be performed.
- **The second step** uses a test called an immunoblot test, commonly, a "western blot" test.
- Results are considered positive only if the EIA/IFA and the immunoblot are both positive.[16,35]

The CDC additionally notes that "new tests may be developed as alternatives to one or both steps of the two-step process. Before CDC will recommend new tests, they must be cleared by the Food and Drug Administration (FDA)."[16]

In the 2019 update concerning the CDC recommendations for serologic diagnosis of LD, they state, "When cleared by FDA for this purpose, serologic assays that utilize EIA rather than western immunoblot assay in a two-test format are acceptable alternatives for the laboratory diagnosis of Lyme disease. Based on the criteria established at the 1994 Second National Conference on Serologic Diagnosis of Lyme Disease, clinicians and laboratories should consider serologic tests cleared by FDA as CDC-recommended procedures for Lyme disease serodiagnosis."[35]

The Infectious Diseases Society of America (IDSA), The American Academy of Neurology (AAN), and The American College of Rheumatology (ACR)

The IDSA, AAN and ACR have published clinical practice guidelines for the prevention, diagnosis, and treatment of LD. The guidelines include the following statements:

- Following a tick bite, "We recommend submitting the removed tick for species identification. (good practice statement).
- We recommend against testing a removed *Ixodes* tick for *B. burgdorferi* (strong recommendation, moderate quality evidence). The presence or absence of *B. burgdorferi* in an Ixodes tick removed from a person does not reliably predict the likelihood of clinical infection.
- We recommend against testing asymptomatic patients for exposure to *B. burgdorferi* following an *Ixodes* spp. tick bite (strong recommendation, moderate-quality evidence).
- In patients with potential tick exposure in a Lyme disease endemic area who have 1 or more skin lesions compatible with erythema migrans, we recommend clinical diagnosis rather than laboratory testing (strong recommendation, moderate quality evidence).
- In patients with 1 or more skin lesions suggestive of, but atypical for erythema migrans, we suggest antibody testing performed on an acute-phase serum sample (followed by a convalescent-phase serum sample if the initial result is negative) rather than currently available direct detection methods such as polymerase chain reaction (PCR) or culture performed on blood or skin samples (weak recommendation, low-quality evidence).

Comment: If needed, the convalescent-phase serum sample should be collected at least 2–3 weeks after collection of the acute-phase serum sample.

- When assessing patients for possible Lyme neuroborreliosis involving either the peripheral nervous system (PNS) or central nervous system (CNS), we recommend serum antibody testing rather than PCR or culture of either cerebrospinal fluid (CSF) or serum (strong recommendation, moderate-quality evidence).
- If CSF testing is performed in patients with suspected Lyme neuroborreliosis involving the CNS, we (a) recommend obtaining simultaneous samples of CSF and serum for determination of the CSF: serum antibody index, carried out by a laboratory using validated methodology, (b) recommend against CSF serology without measurement of the CSF: serum antibody index, and (c) recommend against routine PCR or culture of CSF or serum (strong recommendation, moderate-quality evidence).
- In patients presenting with 1 or more of the following acute disorders: meningitis, painful radiculoneuritis, mononeuropathy multiplex including confluent mononeuropathy multiplex, acute cranial neuropathies (particularly VII, VIII, less commonly III, V, VI, and others), or in patients with evidence of spinal cord (or rarely brain) inflammation, the former particularly in association with painful radiculitis involving related spinal cord segments, and with epidemiologically plausible exposure to ticks infected with *B. burgdorferi*, we recommend testing for Lyme disease (strong recommendation, moderate-quality evidence).
- In patients with typical amyotrophic lateral sclerosis, relapsing-remitting multiple sclerosis, Parkinson's disease, dementia or cognitive decline, or new-onset seizures, we recommend against routine testing for Lyme disease (strong recommendation, low-quality evidence).
- In patients with neurological syndromes other than those listed… in the absence of a history of other clinical or epidemiologic support for the diagnosis of Lyme disease, we recommend against screening for Lyme disease (strong recommendation, low-quality evidence).
- In patients presenting with nonspecific magnetic resonance imaging white matter abnormalities confined to the brain in the absence of a history of other clinical or epidemiologic support for the diagnosis of Lyme disease, we suggest against testing for Lyme disease (weak recommendation, low-quality evidence).
- In patients with psychiatric illness, we recommend against routine testing for Lyme disease (strong recommendation, low-quality evidence).
- In children presenting with developmental, behavioral, or psychiatric disorders, we suggest against routinely testing for Lyme disease (weak recommendation, low-quality evidence).
- In patients with acute myocarditis/pericarditis of unknown cause in an appropriate epidemiologic setting, we recommend testing for Lyme disease (strong recommendation, low-quality evidence).
- In patients with chronic cardiomyopathy of unknown cause, we suggest against routine testing for Lyme disease (weak recommendation, low-quality evidence).

- When assessing for possible Lyme arthritis, we recommend serum antibody testing over PCR or culture of blood or synovial fluid/tissue (strong recommendation, moderate quality of evidence).
- In seropositive patients for whom the diagnosis of Lyme arthritis is being considered but treatment decisions require more definitive information, we recommend PCR applied to synovial fluid or tissue rather than *Borrelia* culture of those samples (strong recommendation, moderate quality of evidence)."

The guideline also made several relevant comments on the above recommendations:

- The guideline commented that knowing tick characteristics (such as "species, life stage, and an assessment of the degree of blood engorgement") is helpful for early guidance, such as antibiotic management.
- "Serologic testing of asymptomatic patients following a tick bite does not help with treatment decisions."
- "Association of Lyme disease with meningitis, cranial neuritis, radiculoneuritis, and other forms of mononeuropathy multiplex is well established…The few systematic studies that have been performed have failed to identify consistent associations between Lyme disease and amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's disease, or Parkinson's disease…These recommendations place a high value on avoiding false positive Lyme disease test results, which can delay appropriate medical evaluation and treatment of other disorders and lead to unnecessary antibiotic exposure and potential side effects."
- "The main disadvantage of this approach the traditional 'two-tiered approach' is that seroreactivity after successfully treated Lyme borreliosis may persist for years, complicating test interpretation in patients with known previous exposure and/or in patients from highly endemic areas where background seroprevalence is substantial. In such patients, after seroreactivity has been demonstrated, synovial fluid or synovial tissue B. burgdorferi PCR may improve diagnostic specificity."[36]

The American College of Rheumatology (ACR)

The ACR also recommends that "the musculoskeletal manifestations of Lyme disease include brief attacks of arthralgia or intermittent or persistent episodes of arthritis in one or a few large joints at a time, especially the knee. Lyme testing in the absence of these features increases the likelihood of false positive results and may lead to unnecessary follow-up and therapy. Diffuse arthralgias, myalgias or fibromyalgia alone are not criteria for musculoskeletal Lyme disease."[37]

Committee on Infectious Diseases of the American Academy of Pediatrics, 32nd/ Edition

The Committee on Infectious Diseases of the American Academy of Pediatrics states that "diagnosis of Lyme disease rests first and foremost on the recognition of a consistent clinical illness in people who have had plausible geographic exposure. Early Lyme disease in patients with erythema migrans is diagnosed clinically on the basis of the characteristic appearance of this skin lesion. Although erythema migrans is not pathognomonic for Lyme disease, it is highly distinctive and characteristic. In areas with

endemic Lyme disease, it is expected that the vast majority of erythema migrans occurring in the appropriate season is attributable to *B burgdorferi* infection."[25]

The AAP report a 2-tier serologic algorithm as the standard testing method for Lyme disease, in which "The initial screening test identifies antibodies to a whole-cell sonicate, to peptide antigen, or to recombinant antigens of B burgdorferi using an enzyme-linked immunosorbent assay (ELISA or EIA) or immunofluorescent antibody (IFA) test. It should be noted that clinical laboratories vary somewhat in their description of this test. It may be described as "Lyme ELISA," "Lyme antibody screen," "total Lyme antibody," or "Lyme IgG/IgM." Many commercial laboratories offer EIA/IFA with reflex to western immunoblot if the first-tier assay result is positive or equivocal. Although the initial EIA or IFA test result may be reported quantitatively, its sole importance is to categorize the result as negative, equivocal, or positive."[25]

Then, "If the first-tier EIA result is negative, the patient is considered seronegative and no further testing is indicated. If the result is equivocal or positive, then a second-tier test is required to confirm the result. There are two options for second tier testing: (1) a western immunoblot, which is the standard 2-tiered testing algorithm; or (2) an EIA test that has been specifically cleared by FDA for use as a second-tier confirmatory test, which is the modified 2-tiered testing algorithm". However, the AAP also reports that "Some assays marketed in the United States have reduced sensitivity for European strains of B burgdorferi. For patients potentially infected in Europe, check with the test provider or laboratory director to select tests that have been validated for this purpose."[25]

The AAP Red Book also delineates for whom and when testing is appropriate.

They caution against the use of serologic testing for Lyme disease in children "without symptoms or signs suggestive of Lyme disease and plausible geographic exposure."

They recommend against western immunoblot testing "the initial EIA or IFA test result is negative or without a prior EIA or IFA test, because specificity of immunoblot diminishes if the test is performed alone."

"No polymerase chain reaction (PCR) test for *B burgdorferi* currently is cleared by the FDA. PCR testing of joint fluid from a patient with Lyme arthritis often yields positive results and can be informative in establishing a diagnosis of Lyme arthritis. The role of a PCR assay on blood is not well established; test results usually are negative in early and late Lyme disease and is not recommended routinely. Yield of PCR testing on cerebrospinal fluid samples from patients with neuroborreliosis is too low to be useful in excluding this diagnosis."

"A number of tests for Lyme disease have been found to be invalid on the basis of independent testing or to be too nonspecific to exclude false-positive results. These include urine tests for *B burgdorferi*, CD57 assay, novel culture techniques, and antibody panels that differ from those recommended as part of standardized 2-tier testing. Although these tests are commercially available from some clinical laboratories, they are not FDA cleared and are not appropriate diagnostic tests for Lyme disease."[25]

Moreover, the interpretation of the results of diagnostic testing can be fraught with difficulties. The notable scenarios are reported below.

"Some patients treated with antimicrobial agents for early Lyme disease never develop detectable antibodies against B burgdorferi; they are cured and are not at risk of late disease. Development of antibodies in patients treated for early Lyme disease does not indicate lack of cure or presence of persistent infection. Ongoing infection without development of antibodies ("seronegative Lyme") has not been demonstrated. Most patients with early disseminated disease and virtually all patients with late disease have antibodies against B burgdorferi. Once such antibodies develop, they may persist for many years. Tests for antibodies should not be repeated or used to assess success of treatment."

"A positive IgM immunoblot result can be falsely positive. The IgM assay is useful only for patients in the first 4 weeks after symptom onset. The IgM immunoblot result should be disregarded (or, if possible, not ordered) in patients who have had symptoms for longer than 4 weeks, or symptoms consistent with late Lyme disease, because false-positive IgM assay results are common, and because most untreated patients with disseminated Lyme disease will have a positive IgG result by week 4 of symptoms."

"Lyme disease test results for B burgdorferi in patients treated for syphilis or other spirochete diseases are difficult to interpret."

"Standardized 2-tier testing can be expected to have positive results in patients with *B mayonii* infection", as "patients with *B mayonii* infection develop a serologic response similar to that of patients infected with B burgdorferi."[25]

National Institute for Health and Care Excellence (NICE)

The NICE recommends diagnosis without laboratory testing in patients with EM. For patients without EM, NICE states to consider using an ELISA test. If this ELISA is positive or equivocal, then an immunoblot may be performed. If both tests are positive, then Lyme disease may be diagnosed.[38]

The NICE also published guidelines in 2019 with the following recommendations:

- "People presenting with erythema migrans are diagnosed and treated for Lyme disease based on clinical assessment, without laboratory testing.
- People with suspected Lyme disease without erythema migrans who have a negative enzyme-linked immunosorbent assay (ELISA) test carried out within 4 weeks of their symptoms starting may have the test repeated 4 to 6 weeks later if Lyme disease is still suspected."[39]

The NICE also produced a diagnostic algorithm with the following recommendations:

- "If Lyme disease is still suspected in people with a negative ELISA who have had symptoms for 12 weeks or more, perform an immunoblot test.
- Carry out an immunoblot test, despite an initial negative ELISA, when there is clinical suspicion of Lyme disease. Diagnose Lyme disease in people with symptoms of Lyme disease and a positive immunoblot test.
- If the immunoblot test for Lyme disease is negative (regardless of the ELISA result) but symptoms persist, consider a discussion with or referral to a specialist, to: review whether further tests may be needed for suspected Lyme disease, for example, synovial fluid aspirate or biopsy, or lumbar puncture for cerebrospinal fluid analysis or consider alternative diagnoses (both infectious, including other tick-borne diseases, and non-infectious).
- Initial testing with a combination IgM and IgG ELISA for Lyme disease should be offered because the evidence generally showed better accuracy (both sensitivity and specificity) for combined tests compared to IgM-only and IgG-only tests. The evidence was best for tests based on purified or recombinant antigens derived from the VlsE protein or its IR6 domain peptide (such as a C6)."

This diagnostic algorithm was primarily based off of NICE's 2018 guidelines.[38]

VI. Applicable State and Federal Regulations

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VII. Applicable CPT/HCPCS Procedure Codes

Procedure codes appearing in medical policy documents are only included as a general reference. This list may not be all inclusive and is subject to updates. In addition, codes listed are not a guarantee of payment.

CPT	Code Description
86617	Antibody; Borrelia burgdorferi (Lyme disease) confirmatory test (eg, Western Blot or immunoblot)
86618	Antibody; Borrelia burgdorferi (Lyme disease)
87475	Infectious agent detection by nucleic acid (DNA or RNA); Borrelia burgdorferi, direct probe technique
87476	Infectious agent detection by nucleic acid (DNA or RNA); Borrelia burgdorferi, amplified probe technique
0041U	Borrelia burgdorferi, antibody detection of 5 recombinant protein groups, by immunoblot, IgM

	Proprietary test: Lyme ImmunoBlot IgM Lab/Manufacturer: IGeneX Inc
0042U	Borrelia burgdorferi, antibody detection of 12 recombinant protein groups, by immunoblot, IgG Proprietary test: Lyme ImmunoBlots IgG Lab/Manufacturer: IGeneX Inc
0316U	Borrelia burgdorferi (Lyme disease), OspA protein evaluation, urine Proprietary test: Lyme Borrelia Nanotrap® Urine Antigen Test Lab/Manufacturer: Galaxy Diagnostics Inc

VIII. Evidence-based Scientific References

1. Barbour A. Microbiology of Lyme disease - UpToDate. In: Mitty J, Steere AC, eds. *UpToDate*. 2023. https://www.uptodate.com/contents/microbiology-of-lyme-disease
2. Hu L. Diagnosis of Lyme disease - UpToDate. In: Mitty J, ed. *UpToDate*. 2023. Accessed 1/8/21. https://www.uptodate.com/contents/diagnosis-of-lyme-disease
3. Mead P, Schwartz A. Epidemiology of Lyme disease In: Steere AC, Hall KK, eds. *UpToDate*. 2024. https://www.uptodate.com/contents/epidemiology-of-lyme-disease
4. Pritt BS, Mead PS, Johnson DKH, et al. Identification of a novel pathogenic Borrelia species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study. *The Lancet Infectious diseases*. May 2016;16(5):556-564. doi:10.1016/s1473-3099(15)00464-8
5. Adeolu M, Gupta RS. A phylogenomic and molecular marker based proposal for the division of the genus Borrelia into two genera: the emended genus Borrelia containing only the members of the relapsing fever Borrelia, and the genus Borreliella gen. nov. containing the members of the Lyme disease Borrelia (Borrelia burgdorferi sensu lato complex). *Antonie van Leeuwenhoek*. Jun 2014;105(6):1049-72. doi:10.1007/s10482-014-0164-x
6. Margos G, Marosevic D, Cutler S, et al. There is inadequate evidence to support the division of the genus Borrelia. *International journal of systematic and evolutionary microbiology*. Apr 2017;67(4):1081-1084. doi:10.1099/ijsem.0.001717
7. Hyde JA. Borrelia burgdorferi Keeps Moving and Carries on: A Review of Borrelial Dissemination and Invasion. *Front Immunol*. 2017;8doi:10.3389/fimmu.2017.00114
8. Bacon RM, Kugeler KJ, Mead PS. Surveillance for Lyme disease--United States, 1992-2006. *Morbidity and mortality weekly report Surveillance summaries (Washington, DC : 2002)*. Oct 3 2008;57(10):1-9.
9. Cook MJ. Lyme borreliosis: a review of data on transmission time after tick attachment. *Int J Gen Med*. 2015;8:1-8. doi:10.2147/ijgm.s73791
10. CDC. Lyme Disease Surveillance and Data. Updated May 15, 2024. https://www.cdc.gov/lyme/data-research/facts-stats/index.html
11. Weitzner E, McKenna D, Nowakowski J, et al. Long-term Assessment of Post-Treatment Symptoms in Patients With Culture-Confirmed Early Lyme Disease. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. Dec 15 2015;61(12):1800-6. doi:10.1093/cid/civ735

12. Marques AR. Laboratory diagnosis of Lyme disease: advances and challenges. *Infectious disease clinics of North America*. Jun 2015;29(2):295-307. doi:10.1016/j.idc.2015.02.005
13. Schriefer ME. Lyme Disease Diagnosis: Serology. *Clinics in laboratory medicine*. Dec 2015;35(4):797-814. doi:10.1016/j.cll.2015.08.001
14. Bunikis J, Barbour AG. Laboratory testing for suspected Lyme disease. *The Medical clinics of North America*. Mar 2002;86(2):311-40.
15. John TM, Taege AJ. Appropriate laboratory testing in Lyme disease. *Cleve Clin J Med*. Nov 2019;86(11):751-759. doi:10.3949/ccjm.86a.19029
16. CDC. Clinical Testing and Diagnosis for Lyme Disease. Updated May 15, 2024. https://www.cdc.gov/lyme/hcp/diagnosis-testing/index.html
17. Halperin JJ. Chronic Lyme disease: misconceptions and challenges for patient management. *Infection and drug resistance*. 2015;8:119-28. doi:10.2147/idr.s66739
18. Waddell LA, Greig J, Mascarenhas M, Harding S, Lindsay R, Ogden N. The Accuracy of Diagnostic Tests for Lyme Disease in Humans, A Systematic Review and Meta-Analysis of North American Research. *PloS one*. 2016;11(12):e0168613. doi:10.1371/journal.pone.0168613
19. CDC. Updated CDC Recommendation for Serologic Diagnosis of Lyme Disease. https://www.cdc.gov/mmwr/volumes/68/wr/mm6832a4.htm
20. ZEUS Scientific. ZEUS Borrelia MTTT™: A paradigm shift in testing for Lyme disease. https://www.zeusscientific.com/what-is-mttt
21. Davis IRC, McNeil SA, Allen W, et al. Performance of a Modified Two-Tiered Testing Enzyme Immunoassay Algorithm for Serologic Diagnosis of Lyme Disease in Nova Scotia. *Journal of Clinical Microbiology*. 2020;58(7):e01841-19. doi:10.1128/jcm.01841-19
22. CCDR. Modified two-tiered testing algorithm for Lyme disease serology: the Canadian context. *Can Commun Dis Rep*. May 7 2020;46(5):125-131. doi:10.14745/ccdr.v46i05a05
23. Wormser GP, Schriefer M, Aguero-Rosenfeld ME, et al. Single-tier testing with the C6 peptide ELISA kit compared with two-tier testing for Lyme disease. *Diagnostic microbiology and infectious disease*. Jan 2013;75(1):9-15. doi:10.1016/j.diagmicrobio.2012.09.003
24. Igenex. Lyme ImmunoBlot. https://igenex.com/wp-content/uploads/LymeImmunoBlot-DataSheet.pdf
25. AAP. Lyme Disease. In: Kimberlin DW, Bernstein HH, Meissner HC, eds. *Red Book: 2021–2024 Report of the Committee on Infectious Diseases 32nd Edition*. American Academy of Pediatrics; 2021:482-489.
26. Igenex. Development of a sensitive PCR-dot blot assay to supplement serological tests for diagnosing Lyme disease. https://igenex.com/wp-content/uploads/Publication_Development_ofa_Sensitive_PCR-dot_Blot_Assay_to_Supplement_Serological_Tests_for_Diagnosing_Lyme_Disease.png.pdf
27. Ghosh R, Joung H-A, Goncharov A, et al. Rapid single-tier serodiagnosis of Lyme disease. *Nature Communications*. 2024/08/20 2024;15(1):7124. doi:10.1038/s41467-024-51067-5
28. Joung HA, Ballard ZS, Wu J, et al. Point-of-Care Serodiagnostic Test for Early-Stage Lyme Disease Using a Multiplexed Paper-Based Immunoassay and Machine Learning. *ACS Nano*. Dec 18 2019;doi:10.1021/acsnano.9b08151
29. Shakir SM, Mansfield CR, Hays ED, Couturier MR, Hillyard DR. Evaluation of a Novel High-Definition PCR Multiplex Assay for the Simultaneous Detection of Tick-Borne

Pathogens in Human Clinical Specimens. *J Clin Microbiol*. Dec 18 2019;doi:10.1128/jcm.01655-19
30. Nigrovic LE, Lewander DP, Balamuth F, et al. The Lyme Disease Polymerase Chain Reaction Test Has Low Sensitivity. *Vector Borne Zoonotic Dis*. Dec 10 2019;doi:10.1089/vbz.2019.2547
31. van Gorkom T, Voet W, Sankatsing SUC, et al. Prospective comparison of two enzyme-linked immunosorbent spot assays for the diagnosis of Lyme neuroborreliosis. *Clin Exp Immunol*. Mar 2020;199(3):337-356. doi:10.1111/cei.13393
32. Sabin AP, Scholze BP, Lovrich SD, Callister SM. Clinical evaluation of a Borrelia modified two-tiered testing (MTTT) shows increased early sensitivity for Borrelia burgdorferi but not other endemic Borrelia species in a high incidence region for Lyme disease in Wisconsin. *Diagnostic microbiology and infectious disease*. Jan 2023;105(1):115837. doi:10.1016/j.diagmicrobio.2022.115837
33. Pratt GW, Platt M, Velez A, Rao LV. Utility of Whole Blood Real-Time PCR Testing for the Diagnosis of Early Lyme Disease. *Am J Clin Pathol*. Sep 2 2022;158(3):327-330. doi:10.1093/ajcp/aqac068
34. Arumugam S, Nayak S, Williams T, et al. A Multiplexed Serologic Test for Diagnosis of Lyme Disease for Point-of-Care Use. *Journal of Clinical Microbiology*. 2019;57(12):10.1128/jcm.01142-19. doi:doi:10.1128/jcm.01142-19
35. Mead P, Petersen J, Hinckley A. Updated CDC Recommendation for Serologic Diagnosis of Lyme Disease. *MMWR Morb Mortal Wkly Rep*. Aug 16 2019;68(32):703. doi:10.15585/mmwr.mm6832a4
36. Lantos PM, Rumbaugh J, Bockenstedt LK, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR): 2020 Guidelines for the Prevention, Diagnosis and Treatment of Lyme Disease. *Clinical Infectious Diseases*. 2021;doi:10.1093/cid/ciaa1215
37. ACR. Choosing wisely: The American College of Rheumatology's top 5 list of things physicians and patients should question. https://escholarship.org/content/qt1kj5v9z2/qt1kj5v9z2.pdf?t=rs2emz&v=lg
38. NICE. Lyme disease. https://www.nice.org.uk/guidance/ng95/chapter/Recommendations
39. NICE. Lyme disease. Quality standard [QS186]. Updated July 10, 2019. https://www.nice.org.uk/guidance/qs186/chapter/Quality-statements

IX. Revision History

Revision Date	Summary of Changes
01/01/2023	Initial Effective Date
06/12/2023	Updated background, guidelines, and evidence-based scientific references. Literature review did not necessitate changes to coverage criteria. Policy edited for clarity and consistency. Name Change-Previously titled Lyme Disease Committee approved: 06/12/2023

05/14/2024	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. Committee approved: 05/14/2024
05/21/2025	Reviewed and Updated: Updated the background, guidelines and recommendations, and evidence-based scientific references. Literature review did not necessitate any modifications to coverage criteria. Committee approved: 05/21/2025