Test Specific Guidelines

Lyme Disease Testing

MOL.CS.332.A
v1.0.2023

Introduction

Lyme Disease testing is addressed by this guideline.

Procedures Addressed

The inclusion of any procedure code in this table is provided for informational purposes and is not a guarantee of coverage nor an indication that prior authorization is required.

Procedure addressed by this guideline	Procedure code
Borrelia burgdorferi, infectious agent detection by nucleic acid (DNA or RNA); direct probe technique	87475
Borrelia burgdorferi, Infectious agent detection by nucleic acid (DNA or RNA), amplified probe technique	87476

What Is Lyme Disease?

Definition

Lyme disease (borreliosis) is caused by a Borrelia bacterial infection following a tick bite from the hard-backed Ixodes tick. It was named after the towns Lyme and Old Lyme in Connecticut after a 1975 investigation of 51 cases with a similar form of arthritis.[1] Borrelia are part of the order spirochaetes, which are spiral-shaped bacteria. There are several subspecies, including B. burgdorferi, B. afezelii, B. garinii, B. spielmanii, and B. bavariensis. In the United States, the most common Lyme disease-causing organism is B. burgdorferi.

Lyme disease incidence is significant, exceeding 30,000 new cases annually in the United States.[2] There are some estimates greater than 300,000 when considering unreported cases.[3] The incidence has increased as the geographic range of the ticks have expanded across the northeastern and upper mid-western states in the U.S., and most recently into Canada. It is expected that climate change will result in further northward expansion of the tick's range.[4]

However, the incidence may be confounded by high rates of misdiagnosis due to the systemic nature of Lyme Disease, and non-specific symptoms. One study

found that 84.1% of a population referred to an Infectious Disease Clinic for Lyme disease were misdiagnosed.[5]

Lyme disease has three stages of infection: early localized, early disseminated, and late disseminated. Disseminated infection can affect multiple organs. While there is a broad spectrum of symptoms and severity, the first signs of infection are a characteristic skin rash with a bullseye appearance (called erythema migrans), fever, and non-specific symptoms like headache and lethargy.[6]

There are several manifestations of Lyme disease that can occur through the three phases of infection:

Neuroborreliosis most commonly manifests with painful meningoradiculitis and lymphocytic meningitis. This may also manifest as facial palsy in many cases, as well as multiple cranial neuropathies.[7] Neuroborreliosis is more common in Europe due to the prevalent Borellia subspecies, B. garinii, which is more frequently associated with neuroborreliosis than the other subspecies.[7]

Lyme carditis, which may present as pericarditis, myocarditis, and/or conduction abnormalities

Lyme arthritis manifests as migratory large joint arthritis and is a hallmark of late disseminated Lyme disease in the U.S.[6]

Fibromyalgia and chronic fatigue syndrome have been associated with chronic Lyme disease (CLD) or post-treatment Lyme disease syndrome (PTLDS). These conditions are characterized by unexplained subjective complaints with similar clinical symptoms as fibromyalgia and chronic fatigue syndrome.[8]

Test Information

Introduction

There are several diagnostic tests available for screening and confirmation of Lyme disease. They have variable sensitivity, specificity, and performance characteristics depending on the stage of the infection and testing matrix.

Serologic tests include enzyme-linked immunosorbent assay (ELISA) to detect IgM or IgG antibodies, immunofluorescence assay (IFA), immunoblot, and confirmatory western blot. Molecular detection of Borrelia organisms is also available in bacterial isolates from culture, blood, and tissue biopsies.

Borrelia Burgdorferi IgG or IgM Antibodies and Borrelia IgG or IgM Antibodies (First-Tier Testing)

This test is performed by enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assay (IFA). There are several commercially available kits. The assays use either whole-cell preparations of B. burgdorferi (for the specific test), Borrelia species, or recombinant antigens, such as C10 peptide, to bind to antibodies present in patient serum. Whole-cell sonicate preparations

result in higher sensitivity due to the presence of multiple antigens, but some of the antigens cross-react with antigens from the host or other pathogens, leading to false positives. Overall, there is lower sensitivity in early stages of Lyme disease.[9]

Confirmatory Assay for Borrelia Burgdorferi Antibody (Second-Tier Testing)

This test is performed by western blot and has higher analytical specificity than ELISA. Patient serum is introduced in a separation gel or strip that has been prepared with antigen extracts and/or recombinant antigens native to B. burgdorferi and electrophoretically separated. Antibodies present in the patient's serum bind to the corresponding antigen bands. The gel is then incubated with a chromogenic substrate to visualize the antigen-antibody complexes as "bands".[9]

Infectious Agent Detection by Nucleic Acid (DNA or RNA); Borrelia Burgdorferi, Direct and Amplified Probe Technique

In direct probe techniques, nucleic acid is extracted from the specimen (isolate, tissue, cerebrospinal fluid (CSF), blood, synovial fluid, etc.) and the target sequence is detected by a reporter molecule, such as an oligonucleotide, deoxyribonucleic acid (DNA) fragment, or plasmid DNA. The reporter molecule has a label attached to generate a signal when hybridized to the target sequence in solution or immobilized on solid support. This technique is increasingly being replaced with amplification methods.

In amplified probe techniques, nucleic acid is extracted from the specimen (isolate, tissue, CSF, blood, synovial fluid, etc.) and then amplified using polymerase chain reaction (PCR). The specific organisms are differentiated through features of the melting curve analysis. However, due to the amplification steps, there is a high risk for exogenous contamination, resulting in false-positive results. Additionally, degradation of the DNA during sample transport, storage, and processing steps can result in false negatives.[10] Standard PCR methods have demonstrated poor sensitivity in testing tissue and body fluids because the infectious agent resides in low number.[11]

Borrelia Burgdorferi, Antibody Detection of (4,5,12) Recombinant Protein Groups, by Immunoblot (IgM, IgG)

The methodology is similar to the western blot, but is differentiated by using recombinant proteins derived from several species of Borrelia to prepare antigen strips, instead of whole blood lysates.[12]

Borreliosis, OspA Protein Biomarker by Nanotrap Capture with Antigen Detection by Western Blot

Outer surface protein A (OspA) antigen is detectable in urine at low concentrations in patients with Lyme disease. The Nanotrap technology employed in this test enriches low-abundance antigen targets by up to 10,000 fold

(galaxydx.com/nanotrap-urine-test-for-lyme-disease), facilitating laboratory detection. Following centrifugation and washing steps, the concentrated antigen eluate is analyzed by western blot.

Guidelines and Evidence

Introduction

This section includes relevant guidelines and evidence pertaining to testing for Lyme disease.

Borrelia Burgdorferi IgG or IgM and Borrelia IgG or IgM

The diagnosis of Lyme disease can be made clinically in individuals with high pre-test probability, obviating the need for serologic testing. However, serologic testing takes on greater importance with later disseminated disease.[13]

Testing for IgM antibodies is only recommended in the first 30 days of infection, after which IgG tests should be used.[14] Serology should not be used for monitoring treatment, as antibodies can persist for several years post-infection. Negative serology does not rule out early infection within six weeks, as false negatives can occur.[1]

In individuals with an atypical presentation, or later disseminated disease, a two-tiered testing approach is recommended. The first tier consists of an ELISA or immunofluorescence assay, and if positive or equivocal, the same sample is tested by the second-tier western blot.[15]

Borrelia Burgdorferi Antibody, Confirmatory Assay

Western blots require interpretation to determine if there are bands present, which is accomplished by skilled technologists or software. To avoid false positives due to variation in band interpretation, the CDC guidelines require that a specific number of bands be present to classify the result as positive:[6]

"It is imperative to avoid interpreting fewer bands as a positive overall result or evidence of infection because antibodies to several antigens are cross-reactive with non-Borrelial antigens. For example, the 41-kDa band indicates reactive antibody against a *B. burgdorferi* flagellin protein. However, this antibody cross-reacts with other bacterial flagellar proteins and was found in 43% of healthy controls in 1 study, including many persons with little or no exposure risk for Lyme disease."

The two-tiered approach to serology testing was recently updated by the CDC to allow FDA-approved enzyme immunoassays (EIA) to replace traditional western blot assays as the confirmatory test.[16] The two-tiered EIA approach consists of the traditional whole-cell sonicate EIA followed by a C6 peptide EIA. Both tiers (ELISA and western blot or EIA) must be positive to classify the final result as positive. The two-tiered approach maximizes test sensitivity and specificity.

Borrelia burgdorferi; Infectious Agent Detection by Nucleic Acid (DNA or RNA)

Molecular detection of B. burgdorferi using PCR-based technology can identify the organism in cases of neuroborreliosis, synovial fluid in cases of Lyme arthritis, and rarely in skin biopsy specimens.

Synovial fluid PCR testing for B. burgdorferi DNA is often positive prior to treatment, but it is not a reliable marker of spirochetal eradication after antibiotic therapy.[17] Intrathecal antibody production is more sensitive than PCR-based CSF detection in individuals with suspected neuroborreliosis.[18] However, PCR may be useful in individuals with short duration of neurological symptoms in the early stage of the infection before emergence of detectable levels of antibodies in CSF.[6]

"Synovial fluid PCR is >75% sensitive for Lyme arthritis and might be useful in conjunction with other synovial fluid analyses to differentiate Lyme arthritis from other arthritides. Comparatively, PCR of CSF is substantially less sensitive, which limits its clinical utility. In 1 US study, PCR testing of CSF yielded positive results for only 38% of patients with early neuroborreliosis and was even less sensitive for late neuroborreliosis."

Infectious Diseases Society of America clinical practice guidelines stated the following regarding testing to be performed on CSF from individuals suspected of having Lyme neuroborreliosis:[19]

"When assessing patients for possible Lyme neuroborreliosis involving either the 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)."

The chemokine CXCL-13 has been proposed as a marker for Lyme neuroborreliosis, however it is elevated in CSF in other infectious and inflammatory disorders, and has not been studied sufficiently to be recommended.[19,20]

Regarding diagnostic testing for Lyme arthritis, the guidelines state:

"When assessing possible Lyme arthritis, we recommend serum antibody testing over PCR or culture of blood or synovial fluid/tissue (strong recommendation, moderate-quality 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 evidence)."

A meta-analysis examining the overall accuracy of diagnostic tests for the detection of Lyme disease reviewed six studies that analyzed bacterial isolation by culture and detection of B. burgdorferi by PCR in blood and tissue biopsies.[1]

"Overall, the sensitivities of PCR studies conducted in North America were lower than those that employed a two-tiered serology diagnostic protocol. Due to the above limitations, bacterial isolation and PCR are not routinely used as diagnostic tools in clinical practise, although bacterial isolation is considered the gold standard to confirm diagnosis."

A novel B. burgdorferi sensu lato genospecies, B. mayonii, was discovered using real-time PCR assay that targets the chromosomal *oppA1* gene. In the first six patients detected with this infection, the two-tier algorithm was only positive in one patient. Until more is understood about the differences in detection rates by the two-tiered testing approach with this infection, PCR may have a role.[21]

"An important issue raised by identification of the novel *B. burgdorferi* sensu lato genospecies is whether existing Lyme borreliosis diagnostic tests can detect infection with this organism…The clinical range of illness must be better defined in additional patients to ensure that physicians can recognise the infection and distinguish it from other tick-borne infections. Many tick-borne pathogens have global distribution, therefore studies are needed to establish the geographic distribution of human beings and ticks infected with the novel *B. burgdorferi* sensu lato genospecies. Finally, clinicians should be aware of the potential role of *oppA1* PCR for diagnosing infection with this novel pathogen."

Borrelia Burgdorferi, Antibody Detection of (4,5,12) Recombinant Protein Groups, by Immunoblot (IgM, IgG)

Detection of B. burgdorferi and tick-borne relapsing fever antibodies by immunoblot using recombinant proteins is the latest addition of Lyme disease serology testing. There are not any independent studies evaluating the accuracy of this method.[12,22]

Borreliosis, OspA Protein Biomarker by Nanotrap Capture with Antigen Detection by Western Blot

There are insufficient clinical studies examining the role of urine Borrelia antigen testing in the diagnosis of Lyme disease and health outcomes. In one relevant validation study, 24/24 patients with erythema migrans tested positive by the nanotrap antigen test; of those patients, 12 were serology positive, 5 were negative, 3 were serology equivocal, and 4 were not tested.[23] Specificity of the nanotrap urine test for later positive serology outcome was 87.5%. In a set of 100 patients being followed for persistent or recurrent Lyme disease, the nanotrap antigen test was positive in 41%.

Criteria

Introduction

This guideline addresses molecular laboratory testing for the diagnosis of Lyme disease.

B. Burgdorferi, Infectious Agent Detection by Nucleic Acid (DNA or RNA)

CPT code(s): 87475, 87476

Medical Necessity Requirements

Nucleic acid detection of B. burgdorferi through direct or amplified methods for the diagnosis of Lyme disease has not demonstrated value that exceeds the two-tier serology testing strategy, and is therefore determined to be not medically necessary.

Nucleic acid detection of B. burgdorferi performed on synovial fluid to inform therapeutic decisions for seropositive individuals in whom a diagnosis of Lyme arthritis is suspected will be considered for reimbursement on a case-by-case basis.

References

Introduction

These references are cited in this guideline.

Waddell LA, Greig J, Mascarenhas M, et al. The accuracy of diagnostic tests for Lyme disease in humans, a systematic review and meta-analysis of North American research. *PLoS one*. 2016;3(11):1-23.

Clark RP, Hu LT. Prevention of Lyme disease and other tick-borne infections. *Infect Dis Clin North Am.* 2008;22(3):381-96.

Hinckley AF, Connally NP, Meek JI, et al. Lyme disease testing by large commercial laboratories in the United States. *Clin Infect Dis*. 2014 Sep 1;59(5):676-81.

Brownstein JS, Holford TR, Fish D. Effect of climate change on lyme disease risk in North America. *Ecohealth*. 2005 Mar;2(1):38-46. doi: 10.1007/s10393-004-0139-x

Kobayashi T, Higgins Y, Samuels R, et al. Misdiagnosis of lyme disease with unnecessary antimicrobial treatment characterizes patients referred to an academic infectious diseases clinic. *Open Forum Infect Dis*. 2019 Jul 1;6(7):1-9.

Moore A, Nelson C, Molins C, Mead P, Schriefer M. Current guidelines, common clinical pitfalls, and future directions for laboratory diagnosis of Lyme disease, United States. *Emerg Infect Dis*. 2016 Jul; 22(7):1169-77.

Ross Russell AL, Dryden MS, Pinto AA, Lovette JK. Lyme disease: diagnosis and management. *Pract Neurol*. 2018;18:455-464.

Van Hout MC. The controversies, challenges and complexities of Lyme Disease: a narrative review. *J Pharm Pharm Sci*. 2018; 21: 429-436.

Lindsay LR, Bernat K, Dibernardo A. Laboratory diagnostics for Lyme disease. *CCDR*. 2014;40(11):209-17.

Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme Borreliosis. *Clin Micro Rev*. 2005 Jul; 18(3):484-509.

Schutzer SE, Body BA, Boyle J, et al. Direct diagnostic tests for Lyme disease. *Clin Infect Dis*. 2019; 68(6): 1052-1057.

Liu S, Cruz ID, Calalo Ramos C, et al. Pilot Study of Immunoblots with Recombinant *Borrelia burgdorefi* Antigens for Laboratory Diagnosis of Lyme Disease. *Healthcare*. 2018;99(6):1-15.

Fallon BA, Pavlicova M, Coffino SW, Brenner C. A comparison of Lyme disease serologic test results from 4 laboratories in patients with persistent symptoms after antibiotic treatment. *Clin Infect Dis*. 2014 Dec 15;59(12):1705-10.

Centers for Disease Control and Prevention. Two-Tiered Testing for Lyme Disease. Available at: https://www.cdc.gov/lyme/healthcare/clinician_twotier.html.

Centers for Disease Control and Prevention (CDC). Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme disease. *MMWR*. 1995 Aug 11;44(31):590-1.

Mead P, Petersen J, Hinckley A. Centers for Disease Control and Prevention (CDC). Updated CDC recommendation for serologic diagnosis of lyme disease. *MMWR Morb Mortal Wkly Rep* [Internet]. 2019 Aug 16;68(32):702-3.

Arvikar SL & Steere AC. Diagnosis and treatment of Lyme arthritis. *Infect Dis Clin North Am*. 2015;29(2):268-280.

Theel ES, Aguero-Rosenfeld ME, Pritt B, Adem PV, Wormser GP. Limitations and confusing aspects of diagnostic testing neurologic Lyme disease in the United States. *J Clin Microbiol*. 2019;57(1):e01406-18.

Lantos PM, Rumbaugh J, Bockenstedt LK, et al. Clinical practice guidelines by the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR): 2020 guidelines for the prevention, diagnosis and treatment of Lyme disease. *Clin Infect Dis*. 2021;72(1):e1-e48.

Raffetin A, Saunier A, Bouiller K, et al. Unconventional diagnostic tests for Lyme borrelosis: a systematic review. *CMI*. 2020; 26:51-59.

Pritt BS, Mead PS, Hoang Jonson DK, et al. Identification of a novel pathogenic Borrelia species causing Lyme borreliosis with unusually high spirochaetaemia: a descriptive study. *Lancet Infect Dis*. 2016 May;16(5):556-564.

Shah JS, Liu S, Du Cruz I, et al. Line Immunoblot assay for tick-borne relapsing fever and findings in patient Sera from Australia, Ukraine and the USA. *Healthcare*. 2019;121(7):1-17.

Magni R, Espina BH, Shah K, et al. Application of Nanotrap technology for high sensitivity measurement of urinary outer surface protein A carboxyl-terminus domain in early stage Lyme borreliosis. *J Transl Med*. 2015;13:346. doi:10.1186/s12967-015-0701-z.Wilde AAM, Semsarian C, Márquez MF, et al. European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) Expert Consensus Statement on the state of genetic testing for cardiac diseases [published online ahead of print, 2022 Apr 4]. *Europace*. 2022;euac030. doi:10.1093/europace/euac030.