A Review of the Centers for Disease Control and Prevention's Guidelines for the Clinical Laboratory Diagnosis of Lyme Disease

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ABSTRACT

Objective: The purpose of this paper is to review information regarding the current guidelines for the clinical laboratory diagnosis of Lyme disease as set forth by the Centers for Disease Control and Prevention (CDC) to chiropractic physicians and to discuss the clinical utility of this testing.

Methods: The CDC's website was reviewed to determine what their current recommendations are for the clinical laboratory testing of Lyme disease.

Results: The CDC's established guidelines recommend the use of a 2-tiered serologic testing algorithm for the evaluation of patients with suspected Lyme disease.

Conclusion: This review provides doctors of chiropractic with information to remain current with the CDC's recommended guidelines for Lyme disease testing because patients may present to their office with the associated signs and symptoms of Lyme disease. (J Chiropr Med 2016;15:272-280)

Key Indexing Terms: *Lyme Disease; Borrelia burgdorferi; Centers for Disease Control and Prevention (US)*

INTRODUCTION

There are misconceptions regarding the clinical laboratory diagnosis of Lyme disease, and clinicians may not be aware of the established guidelines for testing. Because symptoms can be nonspecific, it can be difficult for clinicians to determine when it is appropriate to order Lyme disease testing. Inappropriate testing contributes to increased health care costs. It may also lead to misdiagnosis and cause the patient to be exposed to unnecessary antimicrobial treatment. To use and interpret a laboratory test correctly, the clinician must understand its limitations, its diagnostic sensitivity and specificity, and what the test is specifically measuring or detecting. Patients may seek treatment from a doctor of chiropractic for the joint pain, myalgias, and neurologic symptoms that often accompany Lyme disease. The purpose of this review is to provide a

brief summary of Lyme disease, present the current laboratory test recommendations by the Centers for Disease Control and Prevention (CDC), describe the diagnostic sensitivity and specificity of laboratory tests, and provide interpretation of test results.

METHODS

The CDC's website was reviewed to determine their current recommendations for the clinical laboratory testing of Lyme disease.

RESULTS

The current testing algorithm recommended by the CDC involves 2-tiered serologic testing that detects immunoglobulins in serum against *B burgdorferi* (Fig 1).^{1,2}

DISCUSSION

Historical Review

In 1975, the Connecticut State Health Department was contacted regarding cases of 12 children in the community of Old Lyme who had arthritis that was initially diagnosed as juvenile rheumatoid arthritis. Steere et al first acknowledged "Lyme arthritis" as a separate disease process and

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Fig 1. Centers for Disease Control and Prevention (CDC) recommended laboratory testing algorithm for Lyme disease. (Image reproduced with permission from the CDC. Available from http://www.cdc.gov/lyme/healthcare/clinician_twotier.html.)

Table 1. Arthropod Vectors and Etiologic Agents by Geographic Area⁷

Geography/Region	Vector	Agent ^a
Northeast, Mid-Atlantic, Upper Midwest	<i>Ixodes scapularis</i> , also known as the deer tick or black-legged tick	<i>Borrelia burgdorferi</i>
West Coast	<i>Ixodes pacificus</i>	<i>B burgdorferi</i>
Europe	<i>Ixodes ricinus</i>	<i>B afzelii</i> , <i>B garinii</i>
Asia	<i>Ixodes persulcatus</i>	<i>B afzelii</i> , <i>B garinii</i>

^a Most common Lyme disease–causing species in these geographic areas; other borreliae have been identified as causing Lyme disease or a Lyme disease–like illness or have unknown pathogenicity.³⁷⁻³⁹

speculated that it was transmitted by an arthropod vector. They first described “Lyme arthritis” in published reports in 1977.³

Burgdorfer et al identified the etiologic agent in 1981.⁴ He and his research team were able to isolate spirochetal bacteria from *Ixodes dammini* (*scapularis*) ticks gathered from an endemic area. These spirochetes reacted with immunoglobulins from patients recovering from Lyme disease.⁴

Epidemiology

Lyme disease, also known as Lyme borreliosis, is the most common tick-borne infection in North America.⁵ It is caused by the bacterium *Borrelia burgdorferi*. The disease

is endemic in the Northeast, mid-Atlantic, upper Midwest, and the West Coast in northern California and Oregon. The arthropod vector in the Northeast, mid-Atlantic, and upper Midwest is *Ixodes scapularis* (blacklegged or deer tick), and on the West Coast, *Ixodes pacificus*.⁶ Lyme borreliosis is also endemic in Europe and Asia, although the arthropod vector and etiologic agents differ (Table 1).⁷

Standardized surveillance and reporting of Lyme disease began in 1991 after it became a nationally reportable disease.⁵ From 1992 to 2006, the number of cases increased 101%, and 93% of the cases were from Connecticut, Delaware, Massachusetts, Maryland, Minnesota, New Jersey, New York, Pennsylvania, Rhode Island, and Wisconsin. The majority of the cases occurred in June, July, and August.⁵

Fig 2. Erythema migrans (EM). EM may present as the classical bull's-eye appearance (erythematous lesion with central clearing; top left, posterior right upper arm). It may also appear as a homogeneously erythematous lesion (top right, right popliteal region) or a vesicular lesion (bottom, right posterior shoulder). EM occurs at the site of the tick bite and is commonly seen on the axillary region, popliteal fossa, gluteal region, and hairline, but may present anywhere on the body.¹¹ (Images reproduced with permission from the Centers for Disease Control and Prevention Public Health Image Library [top left: ID #9873 photographer James Gathany, top right ID #14478, bottom ID #14474], and are available at <http://phil.cdc.gov/phil/quicksearch.asp>. Selected Keywords: erythema, migrans.)

Clinical Presentation

The stages of Lyme disease include the early localized stage, early disseminated stage, and late disseminated stage. The early localized stage begins 3 to 30 days after the initial tick bite. A localized, erythematous, circular macule or papule known as erythema migrans (EM) develops during this stage at the site of the tick bite in 70% to 80% of individuals. It expands over the course of days to weeks, sometimes with central clearing to give a bull's-eye appearance.^{8,9} Erythema migrans is the most common clinical manifestation of Lyme disease and is a result of cutaneous infection by *B burgdorferi*.¹⁰ Erythema migrans is ≥ 5 cm across the largest diameter (Fig 2).⁹ Patients with or without EM may also exhibit flulike symptoms, including fatigue, headache, lymphadenopathy, arthralgia, myalgia, low-grade fever, and chills. Those who do not develop EM may only present with flulike symptoms.^{7,8} In this situation, the clinician would have to take into account the season and patient exposure to a tick habitat in an endemic area in order to have a clinical suspicion of Lyme disease.¹¹

If not treated in the early localized stage, the early disseminated stage begins days to weeks after the tick bite,

and infection spreads to other areas. The patient may present with multiple secondary EM, arthritis in the large joints, neurologic involvement, and cardiac involvement manifesting as atrioventricular block.^{7,8} The late disseminated stage begins months to years after the tick bite and occurs in 60% of patients who have not been treated. The patient may present with intermittent attacks of arthritis, and 5% go on to develop chronic neurologic symptoms.⁸

Lyme neuroborreliosis occurs in 5% to 20% of Lyme disease cases in North America.¹² Manifestations include aseptic meningitis, encephalopathy, cranial nerve palsies, and peripheral neuropathies. Peripheral nervous system involvement may manifest as paresthesias or radicular pain (acute polyradiculopathy, brachial or lumbosacral plexopathy, mononeuropathy multiplex). Cranial nerve palsies are the most common manifestation of early (acute) neurologic involvement in the United States.^{10,12} Cranial nerve VII is most commonly affected, and involvement can be bilateral.⁷ Both upper and lower facial asymmetry are evident on evaluation of the muscles of facial expression. Cranial nerves III, IV, V, VI, and VIII can also be affected.

Table 2. Definitions and Terms Regarding the Utility of Clinical Laboratory Testing^{40,41}

Term	Meaning
True positive	The patient has the disease, and the test is positive.
False positive	The patient does not have the disease, and the test is positive.
True negative	The patient does not have the disease, and the test is negative.
False negative	The patient has the disease, and the test is negative.
Diagnostic sensitivity	Capacity of a clinical test to correctly identify those who truly have the disease (true positive).
Diagnostic specificity	Capacity of a clinical test to correctly identify those who truly do not have the disease (true negative).
Description	Generally, tests with a high sensitivity have a low specificity and vice versa. Therefore, it is usually best to screen patients with a test that has a high sensitivity, and then use a second test with a high specificity to identify any false positives.
Predictive value	Based on the prevalence of the disease in a given population and the sensitivity and specificity of the clinical test.
Positive predictive value	The probability that the patient with a positive result actually has the disease. Determined by dividing the number of true positives by the total number of positive results (true positives and false positives).
Negative predictive value	The probability that the patient with a negative result actually does not have the disease. Determined by dividing the number of true negatives by the total number of negative results (true negatives and false negatives).

The patient may present with diplopia, facial paresthesias, hearing loss, or vertigo.^{11,12} These abnormalities can be detected during cranial nerve examination.

Acute neuroborreliosis may cause sudden onset of motor and sensory loss, radicular pain, and intermittent paresthesias. This can mimic spinal nerve root compression but may be differentiated on the basis of a lack of injury history and multiple thoracic dermatome involvement. Glove and stocking sensory deficits are common.¹¹

Aseptic meningitis caused by Lyme disease produces symptoms of intermittent headaches and neck pain. Fever may be absent or low grade. Laboratory results of cerebrospinal fluid analysis are similar to viral meningitis: mild to moderate lymphocytic pleocytosis, mild increase in protein, and normal to mild decrease in glucose.¹²

Acute neuroborreliosis usually resolves within weeks to months even if the patient remains untreated. In about 5% of patients who remain untreated, chronic neuroborreliosis will develop, manifesting as encephalopathy or chronic axonal polyneuropathy. Chronic axonal polyneuropathy usually presents as radicular pain or distal paresthesias.⁷

Lyme arthritis manifests as intermittent migratory monoarticular or oligoarticular arthritis; polyarticular involvement is rare. The knee is most commonly affected. Lyme arthritis develops in about 60% of those who do not receive treatment.¹¹ The joint swelling and pain can spontaneously resolve within weeks to months.¹³ For surveillance purposes, the CDC defines Lyme arthritis as recurrent, brief attacks over the course of weeks or months of objective monoarticular or oligoarticular joint swelling, or chronic monoarticular or polyarticular joint swelling. Cases of chronic progressive arthritis that are not preceded by brief attacks, chronic symmetrical polyarthritis, or arthralgia or myalgia alone are not sufficient to classify as musculoskeletal involvement of Lyme disease.⁹ Other

musculoskeletal symptoms include tenderness of muscles, tendons, and areas surrounding the joints.¹¹

Laboratory Testing

Serologic testing is the only Lyme disease test method approved by the United States Food and Drug Administration (FDA).¹⁴ First-tier testing measures total immunoglobulin M and immunoglobulin G (IgM/IgG) or separate IgM and IgG with an enzyme immunoassay (EIA). Occasionally, immunofluorescence assays (IFA) may be used, although they are becoming increasingly less common for this application.^{1,8,14} Different types of FDA-approved EIAs include enzyme-linked immunosorbent assays and enzyme-linked fluorescent immunoassays.¹⁵ Enzyme immunoassays are quantitative tests¹⁴; they are diagnostically sensitive but not specific, with only about 85% specificity (Table 2).¹⁶ Therefore, false positive EIAs for Lyme disease may occur as a result of the presence of cross-reactive antibodies that occur with other disease conditions, including tick-borne relapsing fever, syphilis, anaplasmosis, bacterial endocarditis, leptospirosis, infectious mononucleosis, some autoimmune diseases, *Helicobacter pylori*, and *Treponema denticola*. Second-tier testing should be able to differentiate Lyme disease from these conditions because it has a higher diagnostic specificity.¹⁵

Further testing is not performed if the first tier test is negative, because this will cause a reduction in the test's diagnostic specificity, which has the potential to yield a false positive result.¹⁶ In the acute phase of the disease, the false negative rate of the EIA is 32%.¹⁷ If the patient has signs and symptoms that are consistent with Lyme disease and have been present for less than 30 days, the clinician may consider testing a convalescent specimen 2 to 4 weeks after the first specimen to allow time for a detectable antibody response to develop. If the first-tier test is positive

or indeterminate/equivocal (result close to the calculated cutoff value, the range between positive and negative), second-tier testing is performed.^{1,18}

Second-tier testing uses an immunoblot test, an IgM and IgG Western blot, which has a high diagnostic specificity.^{1,2,16}

The Western blot detects immunoglobulins to specific antigens of *B burgdorferi*.¹⁹ The antigens are separated on a support medium, and the specific antibody response to these antigens is evaluated (Fig 3). This method is qualitative or semiquantitative and is more subjective because of interpretation of reactive band intensity.¹⁴

During the first 30 days of the disease, an IgM response is produced, typical of the acute phase of an illness; an IgG response generally does not develop until after 30 days.¹ If the patient has had signs and symptoms for ≤ 30 days, IgM and IgG Western blots are performed. If the patient has had signs and symptoms for >30 days, only an IgG Western blot should be performed because a positive IgM Western blot at this stage is likely a false positive.^{1,2,19} Dressler et al observed that after the first few weeks of infection, patients had developed an IgG response.²⁰ Sequential testing is essential for accurate diagnosis. Enzyme immunoassays or IFA should always be done first, and Western blots should only be performed after a positive or equivocal EIA or IFA. Failure to perform testing in sequence will increase the probability of yielding a false positive result because of decreasing the test specificity.^{1,14} The IgM immunoblot is reported as positive only if 2 of 3 specific bands are present: 24 kDa (OspC), 39 kDa (BmpA), and 41 kDa (Fla). The IgG immunoblot is considered positive if 5 of 10 bands are present: 18 kDa, 21 kDa (OspC), 28 kDa, 30 kDa, 39 kDa (BmpA), 41 kDa (Fla), 45 kDa, 58 kDa (not GroEL), 66 kDa, and 93 kDa (Fig 3).² The CDC developed these criteria based on the work of Dressler and Engstrom.^{20,21} A positive or equivocal EIA or IFA with a negative Western blot is reported as negative. A positive or equivocal EIA or IFA with a positive Western blot is reported as positive.⁸

Immunoglobulin M and IgG titers can remain elevated for years after successful treatment, so IgM is not a dependable indicator of reinfection; clinical signs and symptoms must be present.⁷ In cases of suspected neuroborreliosis, cerebrospinal fluid may be evaluated for the presence of antibodies by EIA or IFA and must have a higher titer than the serum specimen.⁹

The idea that EIAs are not sensitive is fairly common but not entirely accurate. Their sensitivity varies depending on

Fig 3. Immunoglobulin G (IgG) Western blot quality control strip demonstrating characteristic bands for *Borrelia burgdorferi*.⁴² Patient test strips are placed next to the control strip for band identification. For an IgG immunoblot to be reported positive, 5 out of 10 specific bands must be present: 18 kDa, 21 kDa (OspC), 28 kDa, 30 kDa, 39 kDa (BmpA), 41 kDa (Fla), 45 kDa, 58 kDa (not GroEL), 66 kDa, and 93 kDa. A positive result indicates detection of IgG antibodies to *B burgdorferi* antigens and reflects exposure, not necessarily active disease. A negative result indicates detection of bands different than the previously stated criteria. A negative result indicates that there were fewer than 5 of 10 of the required bands detected, or no IgG antibodies to the specific antigens were detected. If clinical suspicion of Lyme disease is still high, another specimen may be obtained and tested after an additional 2 to 4 weeks.^{2,42} (Image reproduced with permission from MarDx Diagnostics, Inc., a Trinity Biotech Company.)

the stage of the disease. Patients with EM are positive $\leq 60\%$ of the time with first-tier testing because it can take weeks to develop an immune response in some individuals. However, during the convalescent stage, 80% to 90% are positive.¹⁴ Negative serologic testing does not eliminate the possibility that the patient has Lyme disease.¹⁸ If there is a high clinical suspicion of Lyme disease, and first-tier (or second-tier) testing is negative, a convalescent specimen may be tested. Patients can also be treated empirically, but it is important to keep in mind that antimicrobial therapy may decrease the immune response, leading to false negative serologic test results.^{22,23} Additionally, if first-tier (or second-tier) testing is negative, the clinician may want to consider another diagnosis.¹

The 2-tiered testing algorithm has been validated in retrospective and prospective studies. The diagnostic specificity of 2-tiered testing has been demonstrated to be $\geq 99\%$, and the diagnostic sensitivity is high after EM. The majority (97% to 100%) of patients with Lyme arthritis or chronic neuroborreliosis are positive with serologic testing.¹⁴

Methods for the laboratory detection of Lyme disease include culture, molecular detection, and serologic detection. Culture and polymerase chain reaction (PCR) are useful in research settings but are not routinely used clinically for diagnostic purposes at this time because none of the methods are standardized or approved by the FDA.¹⁴ Use of PCR, however, may be changing, as increasing numbers of laboratories are exploring its clinical use by performing laboratory-specific “in-house” methods. *B burgdorferi* can be cultured in Barbour-Stoenner-Kelly medium, but this method has little diagnostic application because the organism is slow growing and is not often recovered from blood, synovial fluid, or tissue, and therefore has low diagnostic sensitivity.^{14,16}

The use of PCR to detect the DNA of *B burgdorferi* has better diagnostic sensitivity than culture in cases of Lyme arthritis. It is sensitive for skin biopsies of EM and synovial fluid and may be diagnostically useful in patients with treatment-resistant Lyme arthritis.¹⁴ It has a low sensitivity for cerebrospinal fluid and therefore is not a reliable method to use for its evaluation.^{10,14}

Some laboratories offer test methods that have not been validated or approved by the FDA.^{24,25} These include capture assays for antigens in urine, immunofluorescence staining for cell wall-deficient forms of *B burgdorferi*, lymphocyte transformation tests, quantitative CD57 lymphocyte assays, measurement of antibodies in synovial fluid, PCR on inappropriate specimens (blood and urine), IgM or IgG immunoblots without previous EIA or IFA, and interpretation of Western blots using unvalidated criteria.^{25,26} The CDC recommends the 2-tiered testing approach using FDA-approved EIA, IFA, and immunoblot tests.²⁴ Clinicians should verify that testing is performed using validated and FDA-approved methods.²⁵

Laboratory testing for Lyme disease is not recommended if the patient has vague complaints such as fatigue,

headache, arthralgia, and myalgia with no clinical signs of Lyme disease, does not have a history of arthropod vector exposure, or is not from or has not traveled to an endemic area.^{8,27} Testing in these populations would decrease the predictive value of the test (Table 2).¹⁴

Laboratory results must always be interpreted in consideration of the whole clinical picture.⁸ Diagnosis of Lyme disease is usually based on clinical findings, a history of exposure in an endemic area, and relevant serologic testing to support clinical findings.⁷ In the United States, EM is the only sign that can be used to make a diagnosis of Lyme disease without supportive laboratory testing.⁹ For patients with suspected disseminated disease or without EM, laboratory confirmation is required and objective clinical manifestations must be present.²⁷

The clinician should keep in mind that *Ixodes* ticks might also transmit *Anaplasma phagocytophilum* (formerly known as *Ehrlichia phagocytophila*), the causative agent of human granulocytic anaplasmosis (previously called human granulocytic ehrlichiosis), and *Babesia* spp, causative agent of babesiosis. The patient may be coinfecting with either or both of these agents in areas where the organisms are endemic.¹⁰

In a 1996 study, Mitchell, Reed, and Hofkes performed serologic testing on 96 patients with Lyme disease from endemic areas of Wisconsin and Minnesota. They demonstrated that 9.4% of these patients had serologic evidence of coinfection; 5.2% were coinfecting with *Anaplasma phagocytophilum*, 2.1% with *Babesia microti*, and 2.1% with both *A phagocytophilum* and *B microti*.²⁸

Coinfection should be included in the clinician's differential diagnosis if the patient's signs and symptoms are more severe and prolonged than would be expected with Lyme disease, if the patient has a high-grade fever, persistence of flulike symptoms after treatment for Lyme disease, or anemia, thrombocytopenia, or leukopenia without the presence of coexisting conditions that would produce such manifestations.¹⁰ If coinfection is suspected, laboratory testing should be ordered for anaplasmosis and babesiosis. For a discussion of laboratory testing for these infectious diseases, please refer to the CDC's *Tickborne Diseases of the United States: Anaplasmosis*, and *Tickborne Diseases of the United States: Babesiosis*.^{29,30}

Doxycycline, the preferred treatment for Lyme disease, is also effective against anaplasmosis, but not babesiosis. Babesiosis requires treatment with atovaquone plus azithromycin or clindamycin plus quinine.¹⁰ The use of doxycycline is contraindicated in pregnant and lactating women and children aged younger than 8 years. Some patients may have an allergy to doxycycline. In these cases, amoxicillin or cefuroxime axetil can be used to treat Lyme disease. Patients with neurologic or cardiac manifestations may be treated with intravenous ceftriaxone or penicillin.¹⁰

Lyme disease is a nationally notifiable disease.³¹ States and local territories voluntarily report information to the CDC for surveillance purposes to help prevent and control

disease, change public health policy, identify individuals at risk, and monitor disease patterns.³² Refer to the CDC's Nationally Notifiable Diseases Surveillance System for the most current Lyme disease case definition.⁹

States have laws that require certain conditions to be reported to boards of health.³³ The clinician must report any suspected or laboratory confirmed case of Lyme disease to either the local board of health where the disease was diagnosed or to the state health department.³³⁻³⁵ Clinicians should familiarize themselves with their state's reporting requirements and procedures. This information can be found on the local or state board or department of health's website. Clinicians need to inform patients that they are required to report their condition beforehand, in case the patient is contacted by the health department for further information.³⁶ It is the chiropractic physician's responsibility to promptly refer patients to their prescribing primary care provider or infectious disease specialist for treatment with the appropriate antimicrobial agents.

Limitations

The article is meant to provide an overview of the clinical laboratory diagnosis of Lyme disease and does not discuss in detail physical examination findings, differential diagnosis, or pathophysiology. It does not offer specific information regarding the determination of the pretest probability of Lyme disease. In-depth analysis of laboratory assay principles and theories are not discussed. The 2-tiered serologic testing methodology does have limitations, and many argue that use of this algorithm alone is not sufficient for the laboratory evaluation of patients with Lyme disease.

CONCLUSIONS

The CDC recommends the use of a 2-tiered serologic testing algorithm to evaluate patients with suspected Lyme disease. It is important for doctors of chiropractic to be aware of the proper diagnosis and laboratory test ordering protocols for Lyme disease, because patients may present for treatment of associated symptoms. This ensures that the patient receives an accurate and timely diagnosis and will allow for proper treatment to prevent the development of accompanying sequelae.

FUNDING SOURCES AND CONFLICTS OF INTEREST

No funding sources or conflicts of interest were reported for this study.

CONTRIBUTORSHIP INFORMATION

Concept development (provided idea for the research): C.M.M.

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Practical Applications

- Serologic testing is the only Lyme disease test method approved by the United States Food and Drug Administration.
- First tier testing measures total IgM/IgG or separate IgM and IgG with an enzyme immunoassay.
- Further testing is not performed if the first tier test is negative.
- If the first tier test is positive or equivocal, second tier testing should be performed.

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