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Laboratory Diagnosis of Lyme Disease - Advances and Challenges

Adriana R. Marques, MD

Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 10/12C118 10 Center Dr., Bethesda MD 20892

Adriana R. Marques: amarques@niaid.nih.gov

Synopsis

Lyme disease is the most common tick-borne illness in the United States and Europe. Culture for *B. burgdorferi* is not routinely available. PCR can be helpful in synovial fluid of patients with Lyme arthritis. The majority of laboratory tests performed for the diagnosis of Lyme disease are based on detection of the antibody responses against *B. burgdorferi* in serum. The sensitivity of antibody-based tests increases with the duration of the infection, and patients who present very early in their illness are more likely to have a negative result. Patients with erythema migrans should receive treatment based on the clinical diagnosis. The current Centers for Disease Control and Prevention recommendations for serodiagnosis of Lyme disease is a 2-tiered algorithm, an initial enzyme immunoassay (EIA) followed by separate IgM and IgG Western blots if the first EIA test result is positive or borderline. The IgM result is only relevant for patients with illness duration of less than a month. While the 2-tier algorithm works well for later stages of the infection, it has low sensitivity during early infection. A major advance has been the discovery of VlsE and its C6 peptide as markers of antibody response in Lyme disease. Specificity is extremely important in Lyme disease testing, as the majority of tests are being performed in situations with low likelihood of the disease, a situation where a positive result is more likely to be a false positive. Current assays do not distinguish between active and inactive infection, and patients may continue to be seropositive for years. There is a need to simplify the testing algorithm for Lyme disease, improving sensitivity in early disease while still maintaining high specificity and providing information about the stage of infection. The development of a point of care assay and biomarkers for active infection would be major advances for the field.

Keywords

Lyme disease; *Borrelia burgdorferi*; Laboratory diagnosis; Serology

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Introduction

Lyme disease, or Lyme borreliosis, is a multisystem illness caused by the spirochete *Borrelia burgdorferi* and it is the most common tick-borne illness in the United States and Europe. Newly revised estimates from the Centers for Disease Control and Prevention (CDC) suggest that there are likely to be around 300,000 new cases of Lyme disease per year in the United States¹. *B. burgdorferi* is transmitted by the bite of infected ticks of the *Ixodes ricinus* complex. In the United States, most cases of Lyme disease are due to the blacklegged tick (*Ixodes scapularis*), occurring at the mid-Atlantic, northeast, and upper Midwest regions.

B. burgdorferi is a gram-negative bacteria, and has the elongated and spiral shape of the spirochetes². It varies from 10 to 30 µm in length and 0.2 to 0.5 µm in width. It has a linear chromosome and a variable number of circular and linear plasmids³. The *B. burgdorferi* sensu lato group includes at least 20 genospecies⁴. Three genospecies are most commonly associated with human infections: *B. burgdorferi* sensu stricto, which causes disease in North America and Europe, *B. afzelii* and *B. garinii*, which occur in Europe and Asia⁵. Additional genospecies have been demonstrated to at least occasionally cause human disease in Europe (e.g., *B. spielmanii* and *B. valaisiana*).⁵ There is some variation in the clinical presentation depending on the infecting genospecies, with *B. burgdorferi* sensu stricto predominating in arthritis, *B. garinii* in neurologic disease and *B. afzelii* in chronic skin manifestations⁶. Even within the same genospecies, there is variation in presentation and dissemination capability^{7,8}.

For clinical purposes, Lyme disease is divided into early localized, early disseminated and late stages. Lyme disease usually begins with the characteristic skin lesion, erythema migrans (EM), at the site of the tick bite^{9–11}. After several days or weeks, the spirochete may disseminate and patients can develop neurologic, cardiac and rheumatologic involvement^{12–15}. The infection is characterized by low number of bacteria, which can persist in collagen rich tissues. While antibiotic therapy will accelerate resolution of the disease, manifestations can spontaneously regress without antibiotic therapy. The resolution of disease is mediated by immune responses, which control the infection. However, without antibiotic therapy, it can recur and/or new manifestations can appear^{9,16,17}.

The available laboratory methods for the diagnosis of Lyme disease fall into two categories: direct methods to detect *B. burgdorferi*, and indirect methods that detect the immune response against it, mainly the detection of antibodies against *B. burgdorferi*. It is important to recognize that laboratory tests should be ordered and interpreted in the context of the clinical evaluation and the likelihood that the patient has Lyme disease. This chapter reviews the laboratory diagnostics for Lyme disease (with focus on the United States), and discusses current recommendations and new developments in the field.

Direct Methods for Detection of *B. burgdorferi*

Laboratory tests for direct detection of *B. burgdorferi* are hampered by very low numbers of spirochetes in the majority of clinical samples. The lack of sensitive, relatively easy, fast, direct tests for the presence of *B. burgdorferi* is one of the main challenges in the laboratory

diagnosis of Lyme disease. While direct tests for *B. burgdorferi* can sometimes be helpful, none are required for the diagnosis of the disease. The main direct test modalities used are culture and PCR. Histopathology has limited utility, being used mostly to exclude other diseases, and in the evaluation of suspected cases of borrelial lymphocytoma and acrodermatitis chronica atrophicans^{18,19}. Detection of *B. burgdorferi* is difficult and time-consuming due to the extreme scarcity of organisms^{20–23}. Warthin-Starry and modified Dieterle silver stains, focus-floating microscopy, as well as direct and indirect immunofluorescence assays with anti-borrelial antibodies have been used, but can be difficult to interpret and require special expertise and careful use of controls^{24–26}. At present, no antigen assays are recommended for the diagnosis of Lyme disease. A research test for detection of OspA has been used in cerebrospinal fluid²⁷. An assay to detect antigens in urine has been shown to be unreliable²⁸.

Culture

Culture is not a routinely available diagnostic method for the diagnosis of Lyme disease in clinical practice, due to its relatively low sensitivity, long incubation and the requirement of special media and expertise. However, the ability to isolate and culture *B. burgdorferi* is essential in Lyme disease research, and culture remains the gold standard to confirm the diagnosis. Methods that would improve sensitivity and simplify the procedure, so it could be adopted more extensively, are needed.

B. burgdorferi has a limited metabolic capacity and requires a complex growth media for cultivation. Media used for culturing *B. burgdorferi* include variations of the Barbour-Stoenner-Kelly (BSK) medium²⁹ and the modified Kelly–Pettenkofer (MKP) medium³⁰. Cultures are examined using dark-field microscopy or fluorescent microscopy after staining aliquots with acridine orange, but sensitivity is improved by testing aliquots with PCR methods³¹. *B. burgdorferi* replicates slowly and cultures are kept for 8 to 12 weeks before being considered negative³¹.

The probability of culturing *B. burgdorferi* depends on the specimen, the stage of the disease, and the expertise of the laboratory. It may also depend on the genotype³². Antibiotic therapy with agents effective against *B. burgdorferi* (even a single dose) will significantly impact the recovery rate^{33,34}.

Culture of skin biopsies from EM has a sensitivity of 40 to 60%^{30,34–41}. In the US, where disease is caused by *B. burgdorferi* sensu stricto, positive cultures are associated with shorter duration of the disease and smaller lesions^{35,42}. Positive skin biopsy cultures in central Europe (where most of the isolates were *B. afzelii*) were associated with larger lesions (up to about 15 cm of diameter) and increased duration (up to 30 days)⁴³. These findings are likely related to the different *Borrelia* species and the host immune response that eventually controls the infection. *B. afzelii* causes slow-growing EM lesions with few systemic symptoms, while *B. burgdorferi* sensu stricto is associated with more rapidly expanding skin lesions and more systemic symptoms¹¹. Culture is moderately successful in skin biopsies of acrodermatitis chronica atrophicans lesions³⁴.

Culture of 9-ml plasma samples from untreated patients with early and early disseminated infection has a sensitivity of around 40%, which can be increased to 75% by frequently testing culture aliquots with a sensitive PCR. Blood cultures are more likely to be positive in patients with multiple EM^{31,36}. *B. burgdorferi* is seldom cultured from the blood of Lyme disease patients with later manifestations of the disease^{44,45}. Culture of cerebrospinal fluid is rarely positive^{41,46–48}. *B. burgdorferi* has not been reliably cultivated from synovial fluid⁴⁹.

There are serious concerns⁵⁰ regarding a new serum culture assay that claims a high positivity rate⁵¹ and further validation is needed. Results from another culture assay reported as having high positivity rates in patients with chronic disease⁵² could not be replicated^{53,54}.

PCR

In general, sensitivity of PCR assays for detection of *B. burgdorferi* DNA directly in skin or blood samples appears similar to culture, but there is more variation due to methodology, gene targets and primers sets used^{34–38,42,45}. When optimal culture methods are employed, PCR seems to be less sensitive, particularly for plasma samples, which may relate to the smaller sample volume tested in PCR assays³⁶. A new assay using broad-range PCR and electrospray ionization mass spectrometry appears promising⁵⁵. At this point, the main use of PCR assays is for evaluating synovial fluid samples in patients with Lyme arthritis, where *B. burgdorferi* DNA can be detected in up to 70 to 85% of patients^{42,56,57}. A positive PCR may not necessarily mean an infection is active.⁴² Sensitivity of PCR in cerebrospinal fluid samples of patients with early neuroborreliosis is low (10–30%) and even lower in late disease⁵⁸.

Indirect methods

Indirect methods detect the immune response of the host against the causative organism. The majority of laboratory tests performed for Lyme disease are based on detection of the antibody responses against *B. burgdorferi* in serum. Antibody-based assays are the only type of diagnostic testing for Lyme disease approved by the US Food and Drug Administration.

A major problem in laboratory diagnostics of Lyme disease is the appropriate use of tests. About 3.4 million Lyme serologic tests are done in the United States every year⁵⁹, vastly above the estimated number of 300,000 cases of the disease. It is likely that tests are being used in situations where they are not recommended, including ruling out Lyme disease in populations with a low probability of having the disease. The predictive value of a test is determined by its sensitivity, specificity and the prevalence of the disease in the population to be tested. Consequently, in a patient with low probability of disease, a negative test rules out the disease while a positive test is more likely to be a false positive.

To improve the specificity of serologic testing for Lyme disease, a 2-tier approach (Figure 1) was recommended in 1995 by the CDC⁶⁰. The first step uses a sensitive enzyme immunoassay (EIA) or rarely, an indirect immunofluorescence assay (IFA). If the test is negative, there is no further testing. If the test is borderline or positive, the sample is retested using separate IgM and IgG Western blots (WB, also referred to as immunoblots in the literature) as the second step. The WB is interpreted using standardized criteria, requiring at

least two of three signature bands for a positive IgM WB, and 5 of 10 signature bands for a positive IgG WB. The IgM WB results are used only for disease of less than 4 weeks of duration. These recommendations apply to infection acquired in the US, as other species within the *B. burgdorferi* sensu lato complex can cause disease in Europe and Asia.

The use of specialty laboratories offering non-validated Lyme diagnostic tests, including unique interpretation of western blot results, is discouraged. They offer no documented advantage in terms of sensitivity while there is a large decrease in specificity⁶¹. The use of antibody assays in synovial fluid is not recommended⁶². There is little published information about use of western blot in the cerebrospinal fluid for the diagnosis of neuroborreliosis.

The current 2-tier algorithm works relatively well when used as recommended, but there are many areas for improvement. Problems include the low sensitivity during early infection, subjective interpretation of bands, and confusion by health care providers and patients regarding how to interpret results.

Most assays are based on whole cell sonicate (WCS) derived from cultured *B. burgdorferi*. WCS-based assays can have a significant number of false-positive results because of the presence of cross-reactive antigens⁶³. Also, proteins expressed in culture can differ from *in vivo* expressed antigens. An example is the VlsE lipoprotein, which causes a rapid and strong humoral response during infection, while there is minimal VlsE expression in cultured *B. burgdorferi*. Adding VlsE to both 1st and 2nd tier tests has improved their performance⁶⁴. Tests using the C6 peptide (a 26-amino acid peptide derived from invariant region 6 of VlsE) have comparable sensitivity to WCS-based EIAs, with significantly improved specificity, most markedly in patients with other diseases^{64–72}. The C6 ELISA can also be used in patients who acquire the infection in Europe, as it is able to detect antibody responses elicited by other *B. burgdorferi* sensu lato species, and can be used as a stand-alone diagnostic strategy when such cases are evaluated in the US^{70,73}. A variety of other recombinant and synthetic antigens have been evaluated for use in serodiagnosis of Lyme disease, including antigens combining portions of different proteins. Conserved regions of OspC, an antigen recognized early during the course of infection by *B. burgdorferi*, have been explored to develop diagnostic peptides, used as single or as part of multi-peptide assays^{66,68,74–77}.

The sensitivity of antibody-based tests increases with the duration of the infection, and there will be a lag from initial infection until the time when there are sufficient levels of antibodies to be detected. Patients who present very early in their illness are more likely to have a negative result. Less than 50% of the patients with EM are positive at presentation, and these patients should receive treatment based on the clinical diagnosis. Serological tests are most helpful in patients with clinical findings indicating later stages of Lyme disease.

Figure 2 demonstrates how the duration of illness substantially affects the result of antibody-based tests. In a large study comparing the C6 ELISA with a WCS ELISA and the two-tier algorithm⁶⁹, patients with single EM lesions were less likely to be seropositive than patients with multiple EM; and patients in the convalescent phase were more likely to be positive

than patients in the acute phase. The vast majority of patients with Lyme arthritis or late neuroborreliosis were positive (Figure 2A). In another study⁷⁸, less than 50% of patients with a single EM were positive by WCS ELISA or C6 ELISA and only 14% were positive by 2-tier testing when the patients were tested within the first week of illness, but the sensitivity of the tests increased with each weekly time point thereafter (Figure 2B).

As shown in many studies^{64,69,71,72,78}, the additional IgM WB step decreases sensitivity in early disease, the only situation where its use is indicated. Positive IgM results for *Borrelia* can occur in more than 40% of parvovirus B19 infections⁷⁹, has been observed in patients with human granulocytic anaplasmosis⁸⁰, Epstein-Barr virus infections and patients with autoimmune diseases. Additionally, false positive IgM WBs are common in commercial laboratories⁸¹, and there is misinterpretation of “positive” IgM results in those patients with symptoms > 4 weeks. Therefore, there is a need to change the testing algorithm for early Lyme disease, avoiding the use of the IgM WB. Possible strategies include the use of the WCS ELISA followed by the C6 ELISA^{71,82}, the addition of the VlsE band⁶⁴, and the use of multi-peptide assays^{75,76}.

Future developments that are needed include point of care tests. These tests would be particularly useful in evaluating patients with stage 2 manifestations of Lyme disease like facial palsy or carditis. Currently, if these patients do not have other manifestations of Lyme disease or a very suggestive history, the diagnosis may depend on serological tests results resulting in a delay in appropriate therapy.

Current assays do not distinguish between active and inactive infection, and patients may continue to be seropositive for years, including an IgM response, even after adequate antibiotic treatment^{83,84}. Hopefully, with further studies utilizing new, promising immunoassay techniques, a combination of multiple antigens can be developed that will help in early diagnosis, inform on the stage and disease manifestations, and on the presence of active versus past infection^{85–87}.

Intrathecal antibody production

The concomitant analysis of serum and cerebrospinal fluid is used to demonstrate selective production of anti-*B. burgdorferi* antibodies in the central nervous system. Measuring the antibody concentration only in the cerebrospinal fluid can be misleading, as a positive result may be due to passive transfer of antibodies from the serum. Evidence of intrathecal antibody production is considered a gold standard for the diagnosis of Lyme neuroborreliosis in Europe, where the vast majority of the studies originate and where *B. garinii* is the species most often associated with neurologic disease. There are many difficulties in the interpretation of results from these studies, due to the lack of a gold standard, the use of different case definitions, different assays and interpretative criteria, retrospective evaluation, and little comparison among assays and among laboratories. Overall, the sensitivity of intrathecal antibody production in acute Lyme neuroborreliosis is around 50%^{41,46,88–95}. Intrathecal antibody can persist after therapy^{96,97}. While there are few studies, positive intrathecal antibody production seems to be found less frequently in neuroborreliosis patients in the US^{14,27,93}.

CXCL13

CXCL13 is a B lymphocyte chemoattractant chemokine that is increased in the cerebrospinal fluid of patients with acute Lyme neuroborreliosis and may be helpful in certain clinical settings, but its diagnostic value remains to be established^{46,98,99}. At this point, this test is not routinely available to the clinician.

Other Tests

The clinical usefulness of cell proliferation assays, ELISPOT assays, cytokine measurements, complement split products and lymphocyte transformation tests have not been established, and these tests should not be used for the diagnosis of Lyme disease. Natural killer cell measurements (CD57) are not helpful¹⁰⁰.

Xenodiagnosis, using the natural tick vector (*Ixodes scapularis*) to detect evidence of infection in Lyme disease, is an experimental test, and its clinical applications will depend on the results of future studies. Although xenodiagnosis is unlikely to be used in routine practice, it can offer researchers a tool to develop new tests for the disease.

Conclusion

Over the past years, major advances in laboratory testing for Lyme disease have occurred, but there is need for further progress. Improvements of several aspects of the currently recommended testing algorithm are needed. These include making the algorithm simpler, possibly as a single test or procedure, with objective, quantitative data, that is more sensitive in early disease and independent of disease duration. The use of the current IgM Western blot should be avoided, as it decreases the sensitivity in the clinical situations where it is recommended (early Lyme disease), while having lower specificity than tests for IgG antibody generally. There is a need to improve direct methods for detection of *B. burgdorferi*, and to develop accurate, sensitive and rapid diagnostic tests for early Lyme disease, preferably point-of-care tests. No current test can be used to follow the response to antibiotic therapy; the development of biomarkers for active infection would be a major advance for the field.

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References

1. CDC. How many people get Lyme disease?. 2013. <<http://www.cdc.gov/lyme/stats/humanCases.html>>
2. Tilly K, Rosa PA, Stewart PE. Biology of infection with *Borrelia burgdorferi*. Infect Dis Clin North Am. 2008; 22:217–234. v.10.1016/j.idc.2007.12.013 [PubMed: 18452798]
3. Di L, et al. *BorreliaBase*: a phylogeny-centered browser of *Borrelia* genomes. BMC bioinformatics. 2014; 15:233.10.1186/1471-2105-15-233 [PubMed: 24994456]

4. Radolf JD, Caimano MJ, Stevenson B, Hu LT. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nat Rev Microbiol.* 2012; 10:87–99.10.1038/nrmicro2714 [PubMed: 22230951]
5. Franke J, Hildebrandt A, Dorn W. Exploring gaps in our knowledge on Lyme borreliosis spirochaetes--updates on complex heterogeneity, ecology, and pathogenicity. *Ticks and tick-borne diseases.* 2013; 4:11–25.10.1016/j.ttbdis.2012.06.007 [PubMed: 23246041]
6. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. *Lancet.* 2012; 379:461–473.10.1016/S0140-6736(11)60103-7 [PubMed: 21903253]
7. Wormser GP, et al. *Borrelia burgdorferi* genotype predicts the capacity for hematogenous dissemination during early Lyme disease. *J Infect Dis.* 2008; 198:1358–1364.10.1086/592279 [PubMed: 18781866]
8. Hanincova K, et al. Multilocus sequence typing of *Borrelia burgdorferi* suggests existence of lineages with differential pathogenic properties in humans. *PLoS One.* 2013; 8:e73066.10.1371/journal.pone.0073066 [PubMed: 24069170]
9. Steere AC, et al. The early clinical manifestations of Lyme disease. *Ann Intern Med.* 1983; 99:76–82. [PubMed: 6859726]
10. Nadelman RB, et al. The clinical spectrum of early Lyme borreliosis in patients with culture-confirmed erythema migrans. *Am J Med.* 1996; 100:502–508. [PubMed: 8644761]
11. Strle F, et al. Comparison of culture-confirmed erythema migrans caused by *Borrelia burgdorferi* sensu stricto in New York State and by *Borrelia afzelii* in Slovenia. *Ann Intern Med.* 1999; 130:32–36. [PubMed: 9890847]
12. Kindstrand E, Nilsson BY, Hovmark A, Pirskanen R, Asbrink E. Peripheral neuropathy in acrodermatitis chronica atrophicans - a late *Borrelia* manifestation. *Acta Neurol Scand.* 1997; 95:338–345. [PubMed: 9228267]
13. Halperin JJ, Logigian EL, Finkel MF, Pearl RA. Practice parameters for the diagnosis of patients with nervous system Lyme borreliosis (Lyme disease). Quality Standards Subcommittee of the American Academy of Neurology. *Neurology.* 1996; 46:619–627.
14. Logigian EL, Kaplan RF, Steere AC. Chronic neurologic manifestations of Lyme disease. *N Engl J Med.* 1990; 323:1438–1444. [PubMed: 2172819]
15. Steere AC, Schoen RT, Taylor E. The clinical evolution of Lyme arthritis. *Ann Intern Med.* 1987; 107:725–731. [PubMed: 3662285]
16. Steere AC, et al. Erythema chronicum migrans and Lyme arthritis. The enlarging clinical spectrum. *Ann Intern Med.* 1977; 86:685–698. [PubMed: 869348]
17. Steere AC, et al. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three connecticut communities. *Arthritis Rheum.* 1977; 20:7–17. [PubMed: 836338]
18. Tee SI, et al. Acrodermatitis chronica atrophicans with pseudolymphomatous infiltrates. *Am J Dermatopathol.* 2013; 35:338–342.10.1097/DAD.0b013e31826b7487 [PubMed: 23147352]
19. Mullegger RR, Glatz M. Skin manifestations of lyme borreliosis: diagnosis and management. *Am J Clin Dermatol.* 2008; 9:355–368. 962 [pii]. [PubMed: 18973402]
20. De Koning J, Bosma RB, Hoogkamp-Korstanje JA. Demonstration of spirochaetes in patients with Lyme disease with a modified silver stain. *J Med Microbiol.* 1987; 23:261–267. [PubMed: 2438410]
21. de Koning J, Tazelaar DJ, Hoogkamp-Korstanje JA, Elema JD. Acrodermatitis chronica atrophicans: a light and electron microscopic study. *J Cutan Pathol.* 1995; 22:23–32. [PubMed: 7751475]
22. Johnston YE, et al. Lyme arthritis. Spirochetes found in synovial microangiopathic lesions. *Am J Pathol.* 1985; 118:26–34. [PubMed: 3966535]
23. Duray PH. Clinical pathologic correlations of Lyme disease. *Rev Infect Dis.* 1989; 11 (Suppl 6):S1487–1493. [PubMed: 2814170]
24. Eisendle K, Grabner T, Zelger B. Focus floating microscopy: “gold standard” for cutaneous borreliosis? *Am J Clin Pathol.* 2007; 127:213–222. M27475U16356W058 [pii]. 10.1309/3369XXFPEQUNEP5C [PubMed: 17210530]
25. Aberer E, Kersten A, Klade H, Poitschek C, Jurecka W. Heterogeneity of *Borrelia burgdorferi* in the skin. *Am J Dermatopathol.* 1996; 18:571–579. [PubMed: 8989928]

26. Aberer E, Klade H, Hobisch G. A clinical, histological, and immunohistochemical comparison of acrodermatitis chronica atrophicans and morphea. *Am J Dermatopathol*. 1991; 13:334–341. [PubMed: 1928618]
27. Coyle PK, et al. Detection of *Borrelia burgdorferi*-specific antigen in antibody-negative cerebrospinal fluid in neurologic Lyme disease. *Neurology*. 1995; 45:2010–2015. [PubMed: 7501150]
28. Klemmner MS, et al. Intralaboratory reliability of serologic and urine testing for Lyme disease. *Am J Med*. 2001; 110:217–219. [PubMed: 11182109]
29. Pollack RJ, Telford SR 3rd, Spielman A. Standardization of medium for culturing Lyme disease spirochetes. *J Clin Microbiol*. 1993; 31:1251–1255. [PubMed: 8501226]
30. Ruzic-Sabljić E, et al. Comparison of isolation rate of *Borrelia burgdorferi* sensu lato in MKP and BSK-II medium. *Int J Med Microbiol*. 2006; 296 (Suppl 40):267–273. [PubMed: 16530005]
31. Liveris D, et al. Improving the yield of blood cultures from patients with early Lyme disease. *J Clin Microbiol*. 2011; 49:2166–2168.10.1128/JCM.00350-11 [PubMed: 21490189]
32. Xu G, et al. Detection of heterogeneity of *Borrelia burgdorferi* in ixodes ticks by culture-dependent and culture-independent methods. *J Clin Microbiol*. 2013; 51:615–617.10.1128/JCM.03009-12 [PubMed: 23175266]
33. Nadelman RB, et al. Failure to isolate *Borrelia burgdorferi* after antimicrobial therapy in culture-documented Lyme borreliosis associated with erythema migrans: report of a prospective study. *Am J Med*. 1993; 94:583–588. [PubMed: 8506882]
34. Picken MM, et al. A two year prospective study to compare culture and polymerase chain reaction amplification for the detection and diagnosis of Lyme borreliosis. *Mol Pathol*. 1997; 50:186–193. [PubMed: 9350301]
35. Liveris D, et al. Quantitative detection of *Borrelia burgdorferi* in 2-millimeter skin samples of erythema migrans lesions: correlation of results with clinical and laboratory findings. *J Clin Microbiol*. 2002; 40:1249–1253. [PubMed: 11923340]
36. Liveris D, et al. Comparison of five diagnostic modalities for direct detection of *Borrelia burgdorferi* in patients with early Lyme disease. *Diagn Microbiol Infect Dis*. 2012; 73:243–245.10.1016/j.diagmicrobio.2012.03.026 [PubMed: 22571973]
37. O'Rourke M, et al. Quantitative detection of *Borrelia burgdorferi* sensu lato in erythema migrans skin lesions using internally controlled duplex real time PCR. *PLoS One*. 2013; 8:e63968.10.1371/journal.pone.0063968 [PubMed: 23696863]
38. Cerar T, Ruzic-Sabljić E, Glinsek U, Zore A, Strle F. Comparison of PCR methods and culture for the detection of *Borrelia* spp. in patients with erythema migrans. *Clin Microbiol Infect*. 2008; 14:653–658. CLM2013 [pii]. 10.1111/j.1469-0691.2008.02013.x [PubMed: 18558937]
39. Ruzic-Sabljić E, et al. Comparison of isolation rate of *Borrelia burgdorferi* sensu lato in two different culture media, MKP and BSK-H. *Clin Microbiol Infect*. 2014; 20:636–641.10.1111/1469-0691.12457 [PubMed: 24237688]
40. Coulter P, et al. Two-year evaluation of *Borrelia burgdorferi* culture and supplemental tests for definitive diagnosis of Lyme disease. *J Clin Microbiol*. 2005; 43:5080–5084.10.1128/JCM.43.10.5080-5084.2005 [PubMed: 16207966]
41. Ogrinc K, et al. Suspected early Lyme neuroborreliosis in patients with erythema migrans. *Clin Infect Dis*. 2013; 57:501–509.10.1093/cid/cit317 [PubMed: 23667259]
42. Li X, et al. Burden and viability of *Borrelia burgdorferi* in skin and joints of patients with erythema migrans or Lyme arthritis. *Arthritis Rheum*. 2011; 63:2238–2247.10.1002/art.30384 [PubMed: 21590753]
43. Strle F, et al. Clinical characteristics associated with *Borrelia burgdorferi* sensu lato skin culture results in patients with erythema migrans. *PLoS One*. 2013; 8:e82132.10.1371/journal.pone.0082132 [PubMed: 24386087]
44. Maraspin V, Ogrinc K, Ruzic-Sabljić E, Lotric-Furlan S, Strle F. Isolation of *Borrelia burgdorferi* sensu lato from blood of adult patients with borreliac lymphocytoma, Lyme neuroborreliosis, Lyme arthritis and acrodermatitis chronica atrophicans. *Infection*. 2011; 39:35–40.10.1007/s15010-010-0062-8 [PubMed: 21153429]

45. Nowakowski J, et al. Blood cultures for patients with extracutaneous manifestations of Lyme disease in the United States. *Clin Infect Dis*. 2009; 49:1733–1735.10.1086/648076 [PubMed: 19886794]
46. Cerar T, et al. Diagnostic value of cytokines and chemokines in lyme neuroborreliosis. *Clin Vaccine Immunol*. 2013; 20:1578–1584.10.1128/CVI.00353-13 [PubMed: 23945160]
47. Strle F, Ruzic-Sabljic E, Cimperman J, Lotric-Furlan S, Maraspin V. Comparison of findings for patients with *Borrelia garinii* and *Borrelia afzelii* isolated from cerebrospinal fluid. *Clinical infectious diseases*. 2006; 43:704–710. [PubMed: 16912943]
48. Cerar T, et al. Validation of cultivation and PCR methods for diagnosis of Lyme neuroborreliosis. *J Clin Microbiol*. 2008; 46:3375–3379.10.1128/JCM.00410-08 [PubMed: 18716226]
49. Wormser GP, Nadelman RB, Schwartz I. The amber theory of Lyme arthritis: initial description and clinical implications. *Clin Rheumatol*. 2012; 31:989–994.10.1007/s10067-012-1964-x [PubMed: 22411576]
50. Johnson BJ, Pilgard MA, Russell TM. Assessment of new culture method for detection of *Borrelia* species from serum of lyme disease patients. *J Clin Microbiol*. 2014; 52:721–724.10.1128/JCM.01674-13 [PubMed: 23946519]
51. Sapi E, et al. Improved culture conditions for the growth and detection of *Borrelia* from human serum. *International journal of medical sciences*. 2013; 10:362–376.10.7150/ijms.5698 [PubMed: 23470960]
52. Phillips SE, Mattman LH, Hulinska D, Moayad H. A proposal for the reliable culture of *Borrelia burgdorferi* from patients with chronic Lyme disease, even from those previously aggressively treated. *Infection*. 1998; 26:364–367. [PubMed: 9861561]
53. Marques AR, Stock F, Gill V. Evaluation of a new culture medium for *Borrelia burgdorferi*. *J Clin Microbiol*. 2000; 38:4239–4241. [PubMed: 11060098]
54. Tilton RC, Barden D, Sand M. Culture *Borrelia burgdorferi*. *J Clin Microbiol*. 2001; 39:2747. [PubMed: 11446361]
55. Eshoo MW, et al. Direct molecular detection and genotyping of *Borrelia burgdorferi* from whole blood of patients with early Lyme disease. *PLoS One*. 2012; 7:e36825.10.1371/journal.pone.0036825 [PubMed: 22590620]
56. Nocton JJ, et al. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. *N Engl J Med*. 1994; 330:229–234.10.1056/NEJM199401273300401 [PubMed: 8272083]
57. Persing DH, et al. Target imbalance: disparity of *Borrelia burgdorferi* genetic material in synovial fluid from Lyme arthritis patients. *J Infect Dis*. 1994; 169:668–672. [PubMed: 8158048]
58. Mygland A, et al. EFNS guidelines on the diagnosis and management of European Lyme neuroborreliosis. *Eur J Neurol*. 2010; 17:8–16. e11–14.10.1111/j.1468-1331.2009.02862.x [PubMed: 19930447]
59. Hinckley AF, et al. Lyme disease testing by large commercial laboratories in the United States. *Clin Infect Dis*. 2014; 59:676–681.10.1093/cid/ciu397 [PubMed: 24879782]
60. CDC. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *MMWR Morb Mortal Wkly Rep*. 1995; 44:590–591. [PubMed: 7623762]
61. Fallon BA, Pavlicova M, Coffino SW, Brenner C. A comparison of Lyme disease serologic test results from four laboratories in patients with persistent symptoms after antibiotic treatment. *Clin Infect Dis*. 2014; 10.1093/cid/ciu703
62. Barclay SS, Melia MT, Auwaerter PG. Misdiagnosis of late-onset Lyme arthritis by inappropriate use of *Borrelia burgdorferi* immunoblot testing with synovial fluid. *Clin Vaccine Immunol*. 2012; 19:1806–1809.10.1128/CVI.00383-12 [PubMed: 22971779]
63. Gomes-Solecki MJ, et al. Recombinant chimeric *Borrelia* proteins for diagnosis of Lyme disease. *Journal of clinical microbiology*. 2000; 38:2530–2535. [PubMed: 10878038]
64. Branda JA, et al. 2-tiered antibody testing for early and late Lyme disease using only an immunoglobulin G blot with the addition of a VlsE band as the second-tier test. *Clin Infect Dis*. 2010; 50:20–26.10.1086/648674 [PubMed: 19947857]

65. Marques AR, Martin DS, Philipp MT. Evaluation of the C6 peptide enzyme-linked immunosorbent assay for individuals vaccinated with the recombinant OspA vaccine. *J Clin Microbiol.* 2002; 40:2591–2593. [PubMed: 12089281]
66. Bacon RM, et al. Serodiagnosis of Lyme disease by kinetic enzyme-linked immunosorbent assay using recombinant VlsE1 or peptide antigens of *Borrelia burgdorferi* compared with 2-tiered testing using whole-cell lysates. *J Infect Dis.* 2003; 187:1187–1199.10.1086/374395 [PubMed: 12695997]
67. Philipp MT, Marques AR, Fawcett PT, Dally LG, Martin DS. C6 test as an indicator of therapy outcome for patients with localized or disseminated lyme borreliosis. *J Clin Microbiol.* 2003; 41:4955–4960. [PubMed: 14605123]
68. Burbelo PD, et al. Rapid, simple, quantitative, and highly sensitive antibody detection for lyme disease. *Clin Vaccine Immunol.* 2010; 17:904–909.10.1128/CVI.00476-09 [PubMed: 20392886]
69. Wormser GP, et al. Single-tier testing with the C6 peptide ELISA kit compared with two-tier testing for Lyme disease. *Diagn Microbiol Infect Dis.* 2013; 75:9–15.10.1016/j.diagmicrobio.2012.09.003 [PubMed: 23062467]
70. Branda JA, et al. Performance of United States serologic assays in the diagnosis of Lyme borreliosis acquired in Europe. *Clin Infect Dis.* 2013; 57:333–340.10.1093/cid/cit235 [PubMed: 23592827]
71. Branda JA, Linskey K, Kim YA, Steere AC, Ferraro MJ. Two-tiered antibody testing for Lyme disease with use of 2 enzyme immunoassays, a whole-cell sonicate enzyme immunoassay followed by a VlsE C6 peptide enzyme immunoassay. *Clin Infect Dis.* 2011; 53:541–547.10.1093/cid/cir464 [PubMed: 21865190]
72. Steere AC, McHugh G, Damle N, Sikand VK. Prospective study of serologic tests for lyme disease. *Clin Infect Dis.* 2008; 47:188–195.10.1086/589242 [PubMed: 18532885]
73. Wormser GP, et al. Utility of serodiagnostics designed for use in the United States for detection of Lyme borreliosis acquired in Europe and vice versa. *Med Microbiol Immunol.* 2014; 203:65–71.10.1007/s00430-013-0315-0 [PubMed: 24218117]
74. Gomes-Solecki MJ, et al. Recombinant assay for serodiagnosis of Lyme disease regardless of OspA vaccination status. *J Clin Microbiol.* 2002; 40:193–197. [PubMed: 11773115]
75. Porwancher RB, et al. Multiplex immunoassay for Lyme disease using VlsE1-IgG and pepC10-IgM antibodies: improving test performance through bioinformatics. *Clin Vaccine Immunol.* 2011; 18:851–859.10.1128/CVI.00409-10 [PubMed: 21367982]
76. Arnaboldi PM, et al. Outer surface protein C peptide derived from *Borrelia burgdorferi* sensu stricto as a target for serodiagnosis of early lyme disease. *Clin Vaccine Immunol.* 2013; 20:474–481.10.1128/CVI.00608-12 [PubMed: 23365204]
77. Mathiesen MJ, et al. Peptide-based OspC enzyme-linked immunosorbent assay for serodiagnosis of Lyme borreliosis. *J Clin Microbiol.* 1998; 36:3474–3479. [PubMed: 9817857]
78. Wormser GP, et al. Impact of clinical variables on *Borrelia burgdorferi*-specific antibody seropositivity in acute-phase sera from patients in North America with culture-confirmed early Lyme disease. *Clin Vaccine Immunol.* 2008; 15:1519–1522. CVI.00109-08 [pii]. 10.1128/CVI.00109-08 [PubMed: 18716009]
79. Tuuminen T, Hedman K, Soderlund-Venermo M, Seppala I. Acute parvovirus B19 infection causes nonspecificity frequently in *Borrelia* and less often in *Salmonella* and *Campylobacter* serology, posing a problem in diagnosis of infectious arthropathy. *Clin Vaccine Immunol.* 2011; 18:167–172.10.1128/CVI.00367-10 [PubMed: 21106777]
80. Wormser GP, et al. Positive Lyme disease serology in patients with clinical and laboratory evidence of human granulocytic ehrlichiosis. *Am J Clin Pathol.* 1997; 107:142–147. [PubMed: 9024062]
81. Seriburi V, Ndukwe N, Chang Z, Cox ME, Wormser GP. High frequency of false positive IgM immunoblots for *Borrelia burgdorferi* in clinical practice. *Clin Microbiol Infect.* 2012; 18:1236–1240.10.1111/j.1469-0691.2011.03749.x [PubMed: 22369185]
82. Wormser GP, et al. Comparative cost-effectiveness of two-tiered testing strategies for serodiagnosis of lyme disease with noncutaneous manifestations. *J Clin Microbiol.* 2013; 51:4045–4049.10.1128/JCM.01853-13 [PubMed: 24068010]

83. Kalish RA, et al. Persistence of immunoglobulin M or immunoglobulin G antibody responses to *Borrelia burgdorferi* 10–20 years after active Lyme disease. *Clin Infect Dis*. 2001; 33:780–785. CID001718 [pii]. 10.1086/322669 [PubMed: 11512082]
84. Peltomaa M, McHugh G, Steere AC. Persistence of the antibody response to the VlsE sixth invariant region (IR6) peptide of *Borrelia burgdorferi* after successful antibiotic treatment of Lyme disease. *J Infect Dis*. 2003; 187:1178–1186. JID30027 [pii]. 10.1086/374376 [PubMed: 12695996]
85. Chandra A, Wormser GP, Marques AR, Latov N, Alaedini A. Anti-*Borrelia burgdorferi* antibody profile in post-Lyme disease syndrome. *Clin Vaccine Immunol*. 2011; 18:767–771. 10.1128/CVI.00002-11 [PubMed: 21411605]
86. Chandra A, Latov N, Wormser GP, Marques AR, Alaedini A. Epitope mapping of antibodies to VlsE protein of *Borrelia burgdorferi* in post-Lyme disease syndrome. *Clin Immunol*. 2011; 141:103–110. 10.1016/j.clim.2011.06.005 [PubMed: 21778118]
87. Philipp MT, et al. A decline in C6 antibody titer occurs in successfully treated patients with culture-confirmed early localized or early disseminated Lyme Borreliosis. *Clin Diagn Lab Immunol*. 2005; 12:1069–1074. 10.1128/CDLI.12.9.1069-1074.2005 [PubMed: 16148173]
88. Djukic M, et al. Cerebrospinal fluid findings in adults with acute Lyme neuroborreliosis. *J Neurol*. 2012; 259:630–636. 10.1007/s00415-011-6221-8 [PubMed: 21898139]
89. Stanek G, Lusa L, Ogrinc K, Markowicz M, Strle F. Intrathecally produced IgG and IgM antibodies to recombinant VlsE, VlsE peptide, recombinant OspC and whole cell extracts in the diagnosis of Lyme neuroborreliosis. *Med Microbiol Immunol*. 2013; 10.1007/s00430-013-0322-1
90. Henningsson AJ, Christiansson M, Tjernberg I, Lofgren S, Matussek A. Laboratory diagnosis of Lyme neuroborreliosis: a comparison of three CSF anti-*Borrelia* antibody assays. *Eur J Clin Microbiol Infect Dis*. 2013; 10.1007/s10096-013-2014-6
91. van Burgel ND, Brandenburg A, Gerritsen HJ, Kroes AC, van Dam AP. High sensitivity and specificity of the C6-peptide ELISA on cerebrospinal fluid in Lyme neuroborreliosis patients. *Clin Microbiol Infect*. 2011; 17:1495–1500. 10.1111/j.1469-0691.2011.03459.x [PubMed: 21375653]
92. Cerar T, Ogrinc K, Strle F, Ruzic-Sabljic E. Humoral immune responses in patients with Lyme neuroborreliosis. *Clin Vaccine Immunol*. 2010; 17:645–650. 10.1128/CVI.00341-09 [PubMed: 20164248]
93. Steere AC, Berardi VP, Weeks KE, Logigian EL, Ackermann R. Evaluation of the intrathecal antibody response to *Borrelia burgdorferi* as a diagnostic test for Lyme neuroborreliosis. *J Infect Dis*. 1990; 161:1203–1209. [PubMed: 2345301]
94. Halperin JJ, Golightly M. Lyme borreliosis in Bell's palsy. Long Island Neuroborreliosis Collaborative Study Group. *Neurology*. 1992; 42:1268–1270. [PubMed: 1620330]
95. Smouha EE, Coyle PK, Shukri S. Facial nerve palsy in Lyme disease: evaluation of clinical diagnostic criteria. *Am J Otol*. 1997; 18:257–261. [PubMed: 9093686]
96. Martin R, Martens U, Sticht-Groh V, Dorries R, Kruger H. Persistent intrathecal secretion of oligoclonal, *Borrelia burgdorferi*-specific IgG in chronic meningo-radicular myelitis. *J Neurol*. 1988; 235:229–233. [PubMed: 3373242]
97. Hammers-Berggren S, Hansen K, Lebech AM, Karlsson M. *Borrelia burgdorferi*-specific intrathecal antibody production in neuroborreliosis: a follow-up study. *Neurology*. 1993; 43:169–175. [PubMed: 8423881]
98. Bremell D, et al. Cerebrospinal fluid CXCL13 in Lyme neuroborreliosis and asymptomatic HIV infection. *BMC Neurol*. 2013; 13:2. 10.1186/1471-2377-13-2 [PubMed: 23294475]
99. Schmidt C, et al. A prospective study on the role of CXCL13 in Lyme neuroborreliosis. *Neurology*. 2011; 76:1051–1058. 10.1212/WNL.0b013e318211c39a [PubMed: 21422457]
100. Marques A, Brown MR, Fleisher TA. Natural killer cell counts are not different between patients with post-Lyme disease syndrome and controls. *Clin Vaccine Immunol*. 2009; 16:1249–1250. 10.1128/CVI.00167-09 [PubMed: 19515868]

Key Points

- It is difficult to demonstrate *B. burgdorferi* by direct techniques (culture and polymerase chain reaction [PCR]). The spirochete is more easily found in the skin and plasma samples of patients with early disease (erythema migrans), and in the synovial fluid of patients with Lyme arthritis (using PCR).
- The sensitivity of antibody-based tests increases with the duration of the infection. Less than 50% of the patients with erythema migrans are positive at presentation. These patients should receive treatment based on the clinical diagnosis.
- Serological tests are most helpful in patients with clinical findings indicating later stages of Lyme disease.
- Many tests for Lyme disease are being performed in patients with low likelihood to have the disease, a situation where a positive result is more likely to be a false positive.
- The current assays do not distinguish between active and past infection, and patients may continue to be seropositive for years.
- The use of nonvalidated Lyme diagnostic tests is not recommended.

Figure 1. Current CDC Recommendations on Serologic Diagnosis of Lyme Disease: 2-Tier Algorithm

^aBoth IgG and IgM WB results will be reported, but an IgM WB positive result is only significant for patients who have been ill for less than a month. ELISA: Enzyme-linked immunosorbent assay, IFA: Immunofluorescence Antibody Assay) IgM: immunoglobulin M, IgG: immunoglobulin G, WB: Western Blot. Adapted from ⁶⁰

Figure 2. Serological Results, Clinical Presentation and Duration of Illness

Panel A shows rates of seropositivity for the C6 ELISA, WCS ELISA and 2-tier algorithm in relationship to disease presentation and time of sample (acute and convalescent) while panel B compares rates of seropositivity in relationship with duration of disease in patients with a single erythema migrans. EM: erythema migrans, LNB: Lyme neuroborreliosis, WCS: whole cell sonicate. Adapted from ^{69,78}.