The laboratory diagnosis of Lyme borreliosis: Guidelines from the Canadian Public Health Laboratory Network

Canadian Public Health Laboratory Network

Canadian Public Health Laboratory Network. The laboratory diagnosis of Lyme borreliosis: Guidelines from the Canadian Public Health Laboratory Network. Can J Infect Dis Med Microbiol 2007;18(2):145-148.

Lyme borreliosis is uncommonly seen in Canada. Most cases have occurred in close proximity to small geographical areas where infected ticks have become established. Although few cases are seen, thousands of patients are tested yearly. Unless patients are carefully selected and an appropriately sensitive and specific testing algorithm is applied, large numbers of patients without Lyme borreliosis will be incorrectly diagnosed. The Canadian Public Health Laboratory Network has developed the present guidelines to assist physicians in assessing patients for Lyme borreliosis, and to help guide the choice and interpretation of laboratory testing.

Diagnostic de la borréliose de Lyme en laboratoire : Directives du Réseau des laboratoires de santé publique canadien

La borréliose de Lyme s'observe rarement au Canada. La plupart des cas sont survenus dans zones géographiques restreintes où des tiques infectées s'étaient établies. Bien que peu de cas aient été observés, des milliers de patients subissent des tests annuellement. À moins que les patients ne soient sélectionnés avec soin et qu'un algorithme diagnostique sensible et spécifique approprié ne soit appliqué, d'importants contingents de patients indemnes de la borréliose de Lyme risquent de recevoir un diagnostic erroné. Le Réseau des laboratoires de santé publique du Canada a préparé les présentes directives pour aider les médecins à diagnostiquer la borréliose de Lyme chez leurs patients et pour orienter le choix et l'interprétation des analyses de laboratoire.

Key Words: *Borreliosis; Diagnosis; Lyme disease; Serology*

Lyme borreliosis (Lyme disease) is a tick-borne spirochetal infection caused by *Borrelia burgdorferi*. Lyme disease was first identified in North America by Steere et al in 1977 (1). Subsequently, Burgdorfer (2) identified the causative agent, which now bears his name. The two main vectors of *B burgdorferi* in Canada are the blacklegged tick, *Ixodes scapularis*, and the western blacklegged tick, *Ixodes pacificus*. Western blacklegged tick populations are widely distributed in British Columbia, and populations are largest in the lower mainland, on Vancouver Island, and in the Fraser Valley. Established populations of the blacklegged tick (*I scapularis*) are more focal, and are found in a small number of localities along the shores of Lake Erie and Lake Ontario in Ontario, and near Lunenburg and Bedford in Nova Scotia (3,4). *I scapularis* ticks are occasionally found in areas of Canada where populations are not established, and it is presumed that these ticks are introduced into these areas by migratory birds (5). Approximately 12% of these 'introduced' ticks are infected with the Lyme disease agent. Although it is possible to be bitten by an infected tick anywhere in Canada, the chances of being bitten and subsequently infected are considered low in areas where populations are not established (6).

Lyme disease is most often recognized by the development of a characteristic skin rash called erythema migrans (EM) at the site of the tick bite (7,8). The rash begins as a red macule or papule, rapidly enlarges to a diameter of at least 5 cm and sometimes develop central clearing. A small proportion of individuals may not develop EM, or may fail to recognize or report the skin rash. In such patients, the disease may progress to its disseminated form. Most often, the disseminated form involves the heart, nervous system and joints.

Cardiac manifestations of Lyme disease occur in less than 10% of patients (8). Typically, cardiac involvement leads to impaired conduction to the atrioventricular node, resulting in arrhythmias, heart block and syncopal episodes. Although more extensive myocarditis has been described, significant myocardial dysfunction is uncommon.

In North America, the neurological manifestations of Lyme disease most typically include cranial neuropathies, meningitis, radiculoneuropathy, encephalopathy and myelopathy (9,10). Neurological involvement typically begins four to eight weeks after the tick bite and approximately one month after EM. Specific neurological complaints, including headache, photophobia and paresthesias, may be seen in early Lyme disease in the absence of objective evidence of central nervous system inflammation.

The arthritis, which develops in patients with untreated Lyme disease, is typically monoarticular or oligoarticular in nature (8). Objective evidence of arthritis occurs in 60% of patients in North America and within approximately six months after exposure.

Canadian Public Health Laboratory Network, Winnipeg, Manitoba
Correspondence: Dr Theodore Kuschak, Canadian Public Health Laboratory Network, National Microbiology Laboratory, 1015 Arlington Street, Winnipeg, Manitoba R3E 3R2. Telephone 204-789-7045, e-mail Theodore_Kuschak@phac-aspc.gc.ca
Received for publication December 15, 2006. Accepted December 20, 2006

TABLE 1
Laboratory approach for the diagnosis of Lyme borreliosis

Phase of infection	Recommended testing strategy
Erythema migrans, acute phase (seasonal occurrence, tick-established area)	Clinical diagnosis and empirical treatment
Erythema migrans, acute phase (out of season, not a tick-established area)	EIA* – repeat in four weeks if negative; treatment at physician's discretion
Characteristic neurological, cardiac or joint involvement	EIA* – consider polymerase chain reaction of synovial or spinal fluid
No objective findings	Not recommended
Persistent symptoms following recommended treatment†	Not recommended

**Enzyme immunoassay (EIA) with an approved-in-Canada kit and Western immunoblot confirmation; †Reference 23*

Late Lyme disease may develop in individuals whose early infection has gone undetected, or who are not adequately treated for early or disseminated Lyme disease. The persistent form of the disease may present as one of a number of latent neurological syndromes, including meningitis, peripheral and cranial neuritis, and encephalopathy. The encephalopathy may be manifested by a number of nonspecific symptoms, including disturbance of sleep, behavioural changes and headaches.

Chronic arthritis may affect as much as 20% of patients with untreated Lyme disease. Again, the joint involvement is often mono- or oligoarticular. The symmetrical polyarticular pattern often seen with rheumatoid arthritis is not seen with Lyme disease.

The chronic skin changes of acrodermatitis chronicum atrophicans are seen primarily in Europe and infrequently in North America (7). Typically, the skin involvement has an acral distribution, and extremities are most often involved. The skin changes are often violaceous in colour and may occur superimposed on the site of prior EM.

DIAGNOSIS OF LYME DISEASE

The diagnosis of Lyme disease can be made clinically or in conjunction with laboratory test results. When the skin rash (EM) is typical and when a patient has been exposed to an environment where blacklegged ticks are known to be established, the diagnosis can be made on clinical grounds alone. In parts of Canada where adventitious, unestablished populations of blacklegged or western blacklegged ticks have been noted, a clinical diagnosis is more challenging. When the rash is atypical or occurs in circumstances in which exposure to the appropriate vector tick species was unlikely, diagnosis is based on the demonstration of a serological response to *B burgdorferi*. Immunoglobulin (Ig) M antibodies are usually detectable within weeks of the onset of symptoms; however, a significant proportion of patients with EM may not have detectable antibody at the time of initial presentation (11). Furthermore, when patients are treated very early in the course of illness, antibodies may not develop. When an initial antibody determination is negative, it is suggested that a second serum specimen be collected four weeks later.

When patients have a credible possibility of exposure to infected ticks and objective findings are suggestive of any of the known neurological manifestations of Lyme disease or Lyme arthritis, then serological testing is recommended. Because available serological screening tests have limitations to their specificity, screening of patients with nonspecific subjective symptoms is strongly discouraged (12).

Current evidence suggests that commercially available enzyme immunoassays (EIAs) used for the purpose of screening are sufficiently sensitive (beyond the first month of infection). Used alone, EIA tests presently in use lack the specificity necessary to base a diagnosis of Lyme disease on an unconfirmed result. Although newer EIAs offer the promise of improved performance characteristics, neither peptide-based nor recombinant antigen-based EIAs have been approved for use in Canada at this time. Consequently, it is the recommended practice to confirm initial EIA screening test results with a Western blot test (the two-step approach) (13,14). To assure optimal performance characteristics, guidelines for the interpretation of Western blot testing have been developed (15). Within the first four weeks of illness, both IgM and IgG Western blot tests should be performed for confirmation of initially positive EIA screening test results. Beyond the first four weeks of illness, the IgG Western blot alone should be used for confirmation. The MarDx Western blot (Marblot, MarDx Diagnostics Inc, USA) commonly used in Canada adheres to the United States Centers for Disease Control and Prevention (CDC) criteria for a positive Western blot (eg, of the 10 possible designated bands detectable by the IgG test, at least five should be positive). When an IgM Marblot is performed, it is recommended that two of the three designated bands be present. Lyme disease incidence is still low in Canada compared with the eastern United States and very low or zero in most parts of central Canada and in certain parts in western Canada; in this situation, considering the pretest likelihood of disease, the possibility of false-positive Western blot results should not be ignored (16).

B burgdorferi has been recovered from EM lesions, synovial biopsies, and blood and cerebrospinal fluid. Although the culture method is highly specific, the isolation of *B burgdorferi* is expensive, lacks clinical sensitivity, and requires special media, an invasive procedure and possibly up to six weeks of incubation (11,17). The vast majority of positive cultures have been from patients in the early stage of Lyme disease, especially from patients with EM lesions (11). Culture is unavailable in most Canadian centres.

Nucleic acid amplification testing (NAT) has been applied in many of the same settings as cultures (11,18-20). As with cultures, positive tests are most frequently seen in the early stage of the disease. A variety of targets, both chromosomal and plasmid, have been used for polymerase chain reaction (PCR) testing, and protocols vary greatly among laboratories. Positivity rates depend greatly on the epidemiological circumstances, the clinical features, the nature of the specimen submitted, the primer sets and testing conditions, and the experience of the testing laboratory. Although PCR has been used to detect *B burgdorferi* in urine, this procedure lacks both sensitivity and specificity, and is not recommended (11). At this time, NAT should be considered for research purposes only.

Antigen detection has also been used in both spinal fluid and urine. As with NAT, antigen tests cannot be recommended unless their sensitivity and specificity significantly improve (21).

Serological testing for Lyme disease should not be undertaken without a thorough appreciation of the geographic and seasonal setting in which the diagnosis is being considered, as well as an assessment of the likelihood that a specific symptom or symptoms complex is due to Lyme disease. For example, a patient residing in an area where blacklegged ticks are established who presents with a typical bull's eye rash in July should be considered to have Lyme disease until proven otherwise. A negative serological test should not dissuade the clinician from treating empirically and notifying public health officials. On the other hand, a patient with typical findings of multiple sclerosis or chronic fatigue without objective findings is highly unlikely to have Lyme disease, and both the physician and patient should be dissuaded from serological testing. In such settings, the low pretest likelihood of Lyme disease greatly increases the chance of a false-positive result; such false-positive results are often difficult to discount by either the ordering physician or the patient, and these results often lead to unnecessary treatment. Subsequent escalations of treatment often follow treatment failure (16).

Such situations are further complicated when an initial screening test is negative and subsequent Western blot testing is performed. When the initial likelihood of Lyme disease is small and when subsequent initial serological testing is negative, the pre-Western blot probability of Lyme disease is even more remote. Because Western blot testing for Lyme borreliosis is itself associated with false-positive test results, a positive result in the setting described above is usually a false positive (22).

For this reason, the Canadian Public Health Laboratory Network continues to support a two-step approach for the serodiagnosis of Lyme borreliosis. The use of the initial EIA is recommended. If a positive or borderline result is obtained, then it is recommended that a second-step Western blot be performed. When the epidemiological setting is appropriate and when patients have findings suggestive of Lyme disease, this two-step approach should assure that the vast majority of cases of Lyme borreliosis are recognized. If the patient is seen shortly after the onset of infection, then repeated serological testing may be recommended. In patients with suspect neuroborreliosis, additional testing, such as PCR testing of spinal fluid or joint fluid, may be indicated.

CANADIAN PUBLIC HEALTH LABORATORY NETWORK RECOMMENDATIONS

1. The appearance of a typical EM rash occurring in season and with a history of exposure to ticks should be considered an indication for antibiotic treatment, irrespective of the results of serological testing.
2. An EM-like rash occurring out of season and/or after exposition in a Lyme nonendemic area where ticks are not known to be established should be investigated with antibody testing.
   (a) Initial negative serological tests in patients with skin lesions suggestive of EM should have testing repeated after four weeks.
3. Patients with symptoms and signs suggestive of early disseminated or late Lyme disease should be tested for antibodies to *B burgdorferi*.
   (a) Initial testing should include an EIA commercially available and approved for use in Canada.
   (b) Sera that are positive or indeterminate by an EIA should be subjected to Western blot confirmatory testing.
   (c) Sera that are screened negative for antibodies using an EIA should not be subjected to Western blot testing.
4. Western blot tests should be interpreted using criteria set forth by the CDC Working Group.
   (a) Western blot tests that fail to meet all of the criteria set out by the CDC Working Group should be reported as negative; testing may be repeated when it is appropriate to do so.
   (b) The specific banding patterns seen on Western blots should not be reported.
   (c) When serological testing is requested for Lyme borreliosis, and when the initial screening test is positive and the subsequent Western blot confirmatory test is negative, specimens should be reported as 'negative for antibodies to *B burgdorferi*'.
5. Culturing for *B burgdorferi* is a low-yield procedure and is not encouraged; if performed, it should be done only on biopsies from EM lesions and synovial or spinal fluid.
6. There is inadequate evidence to support the use of *B burgdorferi* antigen testing as an adjunct to the diagnosis of Lyme borreliosis.
   (a) The role of NAT (eg, PCR) is limited, and its use should be restricted to patients with objective evidence of joint or central nervous system infection.
   (b) There is inadequate evidence to recommend PCR testing of blood and urine for the diagnosis of Lyme disease.
7. Patients without objective findings suggestive of *B burgdorferi* infection should not be 'screened' for *B burgdorferi* antibodies.
   (a) The diagnosis of Lyme borreliosis should not be based on positive serological tests in the absence of objective findings of infection and a credible epidemiological link to infected ticks.
   (b) Bypass of laboratories that apply the two-step testing procedure (initial EIA followed by Western blot testing) is strongly discouraged.
   (c) Patients should be made aware that antibody testing is subject to false-positive results, and that a positive test in the absence of objective findings and credible exposure histories usually represent false-positive results.
8. The role of antibody testing to monitor the results of therapy has not been established and is therefore not recommended.
9. The role of the microbiology laboratory in the assessment of patients with the persistence of symptoms following antibiotic treatment has not yet been established.

10. In patients in whom tick exposure occur outside of North America, physicians should seek diagnostic advice on testing from a Canadian laboratory with expertise in the diagnosis of Lyme disease.
11. Testing patients suspected of Lyme disease for other tick-associated diseases should not be routinely performed; instead, testing should be based on risk exposure and clinical symptoms.

REFERENCES

1. Steere AC, Malawista SE, Snydman DR, et al. Lyme arthritis: An epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum 1977;20:7-17.
2. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP. Lyme disease – a tick-borne spirochetosis? Science 1982;216:1317-9.
3. Ogden NH, Trudel L, Artsob H, et al. *Ixodes scapularis* ticks collected by passive surveillance in Canada: Analysis of geographic distribution and infection with the Lyme Borreliosis agent *Borrelia burgdorferi*. J Med Entomol 2006;43:600-9.
4. Morshed MG, Scott JD, Fernando K, et al. Distribution and characterization of *Borrelia burgdorferi* isolates from *Ixodes scapularis* and presence in mammalian hosts in Ontario, Canada. J Med Entomol 2006;43:762-73.
5. Morshed MG, JD Scott, Fernando K, et al. Migratory songbirds disperse ticks across Canada, and first isolation of the Lyme disease spirochete, *Borrelia burgdorferi*, from the avian tick, *Ixodes auritulus*. J Parasitol 2005;91:780-90.
6. Ogden NH, Barker IK, Beauchamp G, et al. Investigation of ground level and remote-sensed data for habitat classification and prediction of survival of *Ixodes scapularis* in habitats of southeastern Canada. J Med Entomol 2006;43:403-14.
7. Mullegger RR. Dermatological manifestations of Lyme borreliosis. Eur J Dermatol 2004;14:296-309.
8. Wormser GP. Clinical practice. Early Lyme disease. N Engl J Med 2006;354:2794-801.
9. Oschmann P, Dorndorf W, Hornig C, Schafer C, Wellensiek HJ, Pflughaupt KW. Stages and syndromes of neuroborreliosis. J Neurol 1998;245:262-72.
10. Logigian EL, Kaplan RF, Steere AC. Chronic neurologic manifestations of Lyme disease. N Engl J Med 1990;323:1438-44.
11. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme borreliosis. Clin Microbiol Rev 2005;18:484-509.
12. Nichol G, Dennis DT, Steere AC, et al. Test-treatment strategies for patients suspected of having Lyme disease: A cost-effectiveness analysis. Ann Intern Med 1998;128:37-48.
13. Robertson J, Guy E, Andrews N, et al. A European multicenter study of immunoblotting in serodiagnosis of Lyme borreliosis. J Clin Microbiol 2000;38:2097-102.
14. Engstrom SM, Shoop E, Johnson RC. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. J Clin Microbiol 1995;33:419-27.
15. 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 Morb Mortal Wkly Rep 1995;44:590-1.
16. Bunikis J, Barbour AG. Laboratory testing for suspected Lyme disease. Med Clin North Am 2002;86:311-40.
17. Berger BW, Johnson RC, Kodner C, Coleman L. Cultivation of *Borrelia burgdorferi* from erythema migrans lesions and perilesional skin. J Clin Microbiol 1992;30:359-61.
18. Nocton JJ, Dressler F, Rutledge BJ, Rys PN, Persing DH, Steere AC. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. N Engl J Med 1994;330:229-34.
19. Nocton JJ, Bloom BJ, Rutledge BJ, et al. Detection of *Borrelia burgdorferi* DNA by polymerase chain reaction in cerebrospinal fluid in Lyme neuroborreliosis. J Infect Dis 1996;174:623-7.
20. Carlson D, Hernandez J, Bloom BJ, Coburn J, Aversa JM, Steere AC. Lack of *Borrelia burgdorferi* DNA in synovial samples in patients with antibiotic treatment-resistant Lyme arthritis. Arthritis Rheum 1999;42:2705-9.
21. Klempner MS, Schmid C, Hu L, et al. Intralaboratory reliability of serologic and urine testing for Lyme disease. Am J Med 2001;110:217-9.
22. Dressler F, Whalen JA, Reinhardt BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. J Infect Dis 1993;167:392-400.
23. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: Clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 2006;43:1089-134.