

Appendix

Laboratory Testing for Lyme Disease in Alberta – March 2012

Introduction:

The purpose of this document is to provide health professionals with an overview of the laboratory testing available for Lyme disease and how results should be interpreted. Each case should be evaluated based on the clinical, epidemiological and laboratory evidence and in consultation with an infectious disease specialist.

Background:

Lyme disease (LD) is a tick-borne zoonotic disease occurring in North America, Europe and Asia. Endemic areas in Canada for Lyme disease transmission are associated with established populations of the blacklegged tick, *Ixodes scapularis* in parts of southern Manitoba, southern and eastern Ontario, southwestern Quebec, New Brunswick and Nova Scotia (1), whereas *I. pacificus* is the primary vector in British Columbia (Vancouver Island, the lower mainland and in the Fraser Valley)(1). Outside of these endemic areas, infected ticks can be deposited by migrating birds or companion animals that acquired them from endemic areas. Presently, Alberta is not considered to be an endemic area for Lyme disease, although a small number of ticks infected with *B. burgdorferi* have been collected off dogs through an on-going passive surveillance program.

The three genospecies causing Lyme disease are *B. burgdorferi*, *afzelii* and *garinii* (collectively referred to as *B. burgdorferi sensu lato*). While all three genospecies are found in Europe and Asia, only *Borrelia burgdorferi* (referred to as *B. burgdorferi sensu stricto* (s.s)) is endemic to North America. Both *B. afzelii* and *B. garinii* are more commonly associated with Lyme disease in Asia and parts of Europe and present with clinical manifestations different to those caused by *B. burgdorferi*. Recently *B. bavariensis* (previously *B. garinii* OspA serotype 4) and *B. spielmanii* have been recognized as human pathogens, whereas the status of *B. lusitaniae*, *valaisiana* and *bissetii* still remains inconclusive (2).

Testing for Lyme disease:

Both serologic and molecular assays [polymerase chain reaction (PCR)] can detect the three genospecies of LD. For PCR testing, the clinical indications and sample type are very restricted [see PCR testing (Table 1a inset in Table 1)] and the laboratory must be contacted, prior to sample collection.

Antibody Screening:

Antibody detection and confirmation follows a two-tiered approach in keeping with the recommendations of the Public Health Agency of Canada (PHAC) and the U.S. Centers for Disease Control and Prevention (CDC), to prevent against the possibility of reporting false-positives as cases of confirmed infections (3,4,5).

Commencing March 23, 2012, the ProvLab will test serum samples in the Lyme C6 enzyme immunoassay (EIA/ELISA) that detects both IgM and IgG antibody to *B. burgdorferi*, *afzelii* and *garinii*, the three genospecies of Lyme disease, with equal sensitivity. However the assay cannot distinguish between them. Positive and equivocal (indeterminate) samples are referred to the National Microbiology Laboratory (NML) for confirmatory testing and genospecies identification by the Western Blotting assay.

Note: Travel history is obligatory as the Western Blot assay for *B. garinii* and *afzelii* is only performed if travel outside of North America is provided: there is no serologic cross-reactivity, between these three genospecies, by the individual Western Blot assays.

Table 1: Laboratory Tests for Lyme disease

Test Name	Test Performance/Indication	Antibody Response
Lyme C6 IgM/IgG Enzyme immunoassay (EIA/ELISA) for serum [Implemented March 23, 2012]	<ul style="list-style-type: none"> Used to screen for LD antibody at ProvLab in suspected cases Detects all three genospecies of <i>Borrelia</i> but cannot distinguish between them Cross reacting antibodies may cause false positive reactions in persons with syphilis, HIV infection, infectious mononucleosis, lupus or rheumatoid arthritis 	<ul style="list-style-type: none"> IgM antibodies to LD generally appear within two to four weeks of erythema migrans (EM) onset and peak around six weeks. IgG antibodies appear within four to six weeks of EM onset and peak around two to three months. Less than 13% of patients with an EM of seven days duration will test positive; approximately 48% will be positive if the EM is present for seven to 14 days and more than 90% will test positive if the EM is greater than 14 days duration.(6) The majority of patients treated effectively shortly after the appearance of EM, will abort a detectable serologic response and repeat testing to document a seroconversion will be futile.(7)
Testing referred to or only available from National Microbiology Laboratory (NML)		
Lyme Disease IgG antibody in CSF	<ul style="list-style-type: none"> Only performed at NML in <u>strongly suspected</u> cases of neuroborreliosis Patient must be confirmed serologically positive for LD Requires paired serum and CSF accompanied by total IgG and albumin concentrations for both specimens 	<ul style="list-style-type: none"> The determination of antibodies in CSF has an advantage over serological testing of serum alone, since cross-reacting antibodies are rarely present in the CSF.
Lyme Disease IgM Western Blot	<ul style="list-style-type: none"> <u>Only</u> performed on sera that test positive/equivocal in the screening assay at ProvLab Only detects antibody to <i>Borrelia burgdorferi</i> Turnaround time is approximately 10 days from receipt at NML 	<ul style="list-style-type: none"> IgM antibodies usually decline to undetectable levels after four to six months (8). However, in some patients a longstanding IgM response is detectable despite effective treatment or historic asymptomatic exposure (9). Hence, detection of IgM antibody alone should not be used as the sole basis to classify a recent exposure, in the absence of appropriate clinical manifestations and symptoms. Initiation of antibiotic treatment early in the course of LD will result in decreased antibody production which in turn will affect the interpretation of the Western Blot.(7)

Test Name	Test Performance/Indication	Antibody Response
Testing referred to or only available from National Microbiology Laboratory (NML)		
Lyme Disease IgG Western Blot	<ul style="list-style-type: none"> • <u>Only</u> performed on sera that test positive/equivocal in the screening assay at ProvLab • Only detects antibody to <i>Borrelia burgdorferi</i> • Turnaround time is approximately 10 days from receipt at NML 	<ul style="list-style-type: none"> • IgG antibody appears soon after the initial IgM antibody response, although it is generally lower in the first few weeks, becoming maximal months later especially in untreated individuals with chronic manifestations of Lyme disease.(8) • Initiation of antibiotic treatment prior to testing may result in decreased antibody production which will affect the interpretation of the Western Blot.(7) • Once IgG antibodies have developed, they can remain detectable for prolonged periods despite adequate treatment.(10)
<i>Borrelia garinii</i> & <i>B. afzelli</i> Western Blot	<ul style="list-style-type: none"> • <u>Only</u> performed on sera that test positive/equivocal in the screening assay at ProvLab • Only detects IgG antibody to <i>B. garinii</i> or <i>afzelli</i>; an IgM-specific Western Blot is not available • Turnaround time is approximately 10 days from receipt at NML 	<ul style="list-style-type: none"> • Travel history <u>is obligatory</u> as these Western Blot assays are only performed if travel outside of North America is provided, as these genospecies are not endemic to this continent. • <u>No serologic cross-reactivity</u>, by the Western blot assay, between the three genospecies.

Test Name	Test Performance/Indication	Antibody Response															
Lyme Disease Polymerase Chain Reaction (PCR) on CSF, synovial fluid, and skin	<ul style="list-style-type: none"> Performed at NML Higher sensitivity than culture Molecular testing is only helpful in selected UNTREATED circumstances, described below, as the yield is generally low: <p>Table 1a: Sensitivity of PCR detection of LD genospecies in different samples (11,13).</p> <table border="1" data-bbox="426 537 1008 1065"> <thead> <tr> <th>Specimen source</th> <th>Sensitivity (%)</th> <th>Comments</th> </tr> </thead> <tbody> <tr> <td>Skin (EM, acrodermatitis chronica atrophicans)</td> <td>50-70</td> <td>Punch biopsy from bite area in Viral Transport medium</td> </tr> <tr> <td>CSF (neuroborreliosis, stage II)</td> <td>10-30</td> <td>Two mL of dedicated CSF</td> </tr> <tr> <td>Synovial fluid (arthritis)</td> <td>50-70</td> <td>At least two mL in a sterile container</td> </tr> <tr> <td>Blood (all stages)</td> <td>10-18</td> <td>Not available</td> </tr> </tbody> </table>	Specimen source	Sensitivity (%)	Comments	Skin (EM, acrodermatitis chronica atrophicans)	50-70	Punch biopsy from bite area in Viral Transport medium	CSF (neuroborreliosis, stage II)	10-30	Two mL of dedicated CSF	Synovial fluid (arthritis)	50-70	At least two mL in a sterile container	Blood (all stages)	10-18	Not available	<ul style="list-style-type: none"> Patients in the acute phase of LD, namely within the first seven days with an EM, and likely exposure to LD, are candidates for detection of the organism by PCR on a punch biopsy of the skin.(11) Some authorities recommend that the punch biopsy is taken from the margin of the EM of the tick bite site whereas others have found the presence of the spirochete at the site of the bite and within the area of the rash.(12) Available from the NML by special request, <u>ProvLab must be contacted prior to submission of samples.</u> All samples submitted for molecular testing must have a companion blood sent for serologic testing, specifically for patients with a provisional diagnosis of neuroborreliosis and/or arthritis, to verify that there is serologic evidence of disease. Comparative studies show that patients with acrodermatitis chronica atrophicans (ACA), caused by <i>B. afzelii</i>, are the most likely to test positive in skin samples, compared with the two other genospecies.(13) Effective treatment also results in loss of viability to culture the organism from the skin despite the presence of the rash (14), although the higher sensitivity of molecular tests may detect residual genomic fragments of the organism. Despite adequate treatment, a sub category of patients will still have residual signs and symptoms, which is due to an autoimmune mechanism rather than an on-going infectious process. (15,16) Molecular testing is of no value in these cases.
Specimen source	Sensitivity (%)	Comments															
Skin (EM, acrodermatitis chronica atrophicans)	50-70	Punch biopsy from bite area in Viral Transport medium															
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Culture on CSF, synovial fluid and skin	<ul style="list-style-type: none"> Not routinely available from NML 	<ul style="list-style-type: none"> The sensitivity of isolating <i>B. burgdorferi</i> from EM lesions, joints, blood and CSF via culture, although possible is variable and largely superseded by PCR (11). 															
Lyme Urine Antigen Test (LUAT) & Lymphocyte Transformation Test (LTT)	<ul style="list-style-type: none"> Not available from NML 	<ul style="list-style-type: none"> No compelling or convincing scientific data to support the value of these tests in making a clinical diagnosis.(17) 															

Table 3: Clinical interpretations based upon the results of the screening and confirmatory assays for Lyme disease

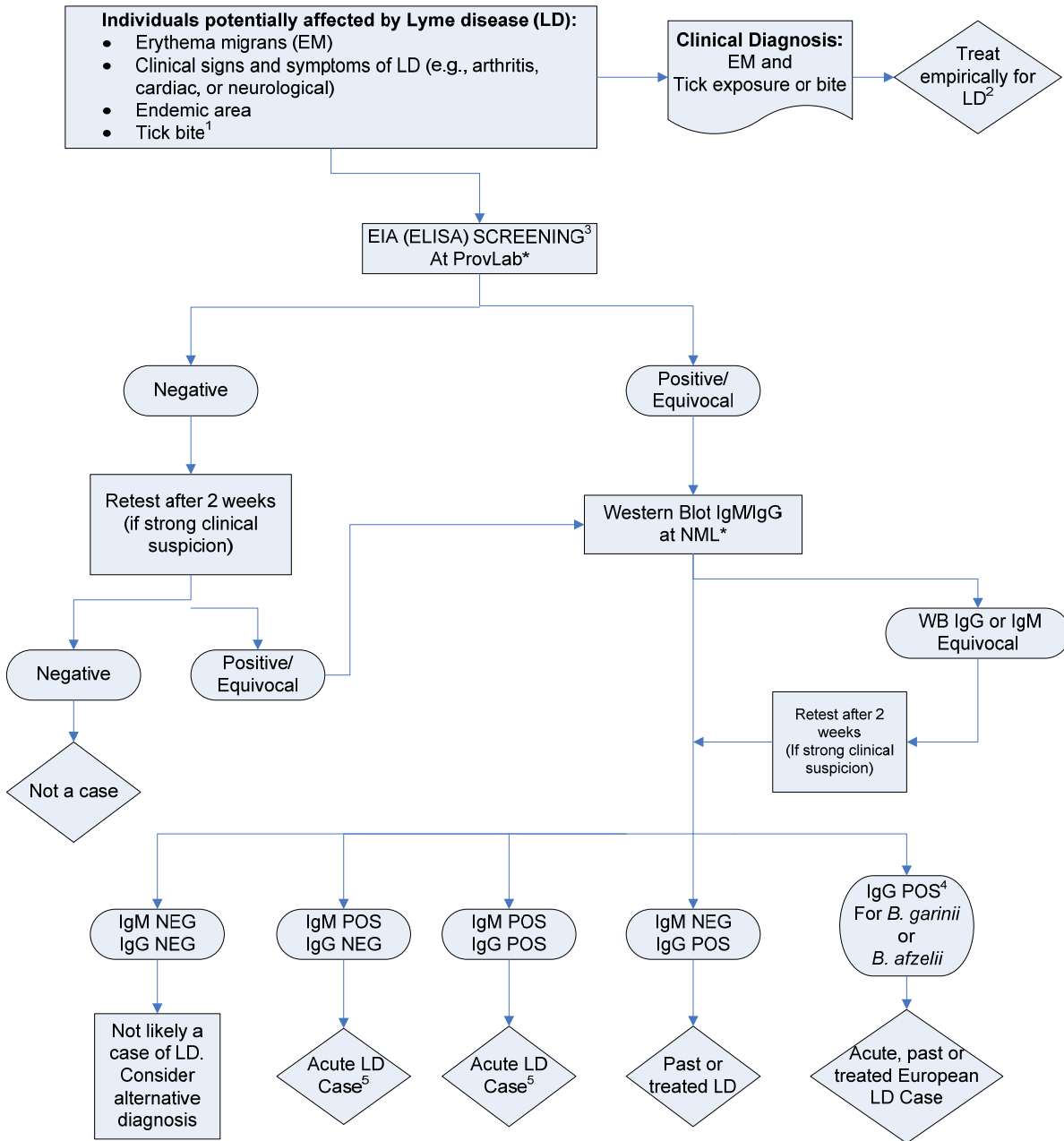
Lyme C6 ¹ EIA/ELISA IgM/IgG Antibody	WB IgM ²	WB IgG ²	Interpretation	Comments
NEGATIVE	-	-	Likely not a case of Lyme disease (LD)	<ul style="list-style-type: none"> If clinical suspicion is high and the patient is not treated, retest after 2 weeks. If negative on retest then unlikely to be LD. Negative sera <u>are not</u> referred to NML for confirmatory testing by Western Blot (WB).
POSITIVE/ EQUIVOCAL ³	NEGATIVE/ EQUIVOCAL	NEGATIVE/ EQUIVOCAL	Likely not a case of Lyme disease (LD)	<ul style="list-style-type: none"> If clinical suspicion is high and the patient is not treated retest after 2-3 weeks. Serum will be referred to NML for confirmatory testing by WB. In areas of low incidence, such as Alberta, cross-reactivity with other unrelated bacterial species can occur. Patient should be evaluated by an infectious disease specialist before considering treatment options, prior to the results of confirmatory testing.
POSITIVE/ EQUIVOCAL	POSITIVE	NEGATIVE	Acute Lyme disease infection or possible false-positive IgM	<ul style="list-style-type: none"> A positive IgM antibody result alone, in the absence of compatible epidemiologic and clinical signs and symptoms, is highly likely to be a false-positive finding. Retest the patient no sooner than two weeks after the first serum. If the patient is still only IgM antibody positive this is indicative of a false-positive finding.
POSITIVE/ EQUIVOCAL	POSITIVE	POSITIVE	Acute Lyme disease infection	<ul style="list-style-type: none"> A positive IgM result four weeks or more after the onset of symptoms should be considered a false-positive.(3,18)
POSITIVE/ EQUIVOCAL	NEGATIVE	POSITIVE	Past or treated Lyme disease infection	
Testing for European Strains (<i>B. garinii</i> and <i>B. afzelii</i>) of Lyme Disease				
POSITIVE/ EQUIVOCAL	Not available	POSITIVE for <i>B. garinii</i> or <i>B. afzelii</i>	Acute, chronic, past or treated LD infection Clinical presentation or history required to stage disease	<ul style="list-style-type: none"> Travel history to Europe / Asia is required for this test to be performed.

¹ The Lyme C6 screening EIA detects all three genospecies (*B. burgdorferi*, *afzelii* & *garinii*) causing Lyme disease, whereas the previous screening EIA was mainly limited to *B. burgdorferi* antibody detection. The change was implemented on March 23, 2012.

² Western Blot is specific for *B. burgdorferi* (*sensu stricto*)

³ EQUIVOCAL = INDETERMINATE and POSITIVE = REACTIVE

Laboratory testing for Lyme Disease and Interpretation, Alberta



¹ If a tick is available, tick speciation done at ProVLab may take up to two weeks. Ticks identified as Ixodes spp. are sent to NML to be tested for LD. Identification of LD in a tick does not mean that an individual is also infected with LD. Also, there may be false negative so treatment should not be delayed waiting for the results of tick testing.
² In consultation with an infectious disease specialist.
³ The Lyme C6 screening EIA detects all three genospecies (*B. burgdorferi*, *afzelii* & *garinii*) causing Lyme disease, whereas the previous screening EIA was mainly limited to *B. burgdorferi* antibody detection. The change was implemented on March 23, 2012.
⁴ The current WB IgM test will not detect European Lyme genospecies. Staging should be based on the patient's clinical and epidemiological history.
⁵ <4 weeks after onset of disease IgM in the absence of IgG is indicative of recent infection. >4 weeks after onset of disease, IgM should not be used in the evaluation of clinical disease as it is likely a false positive

*ProVLab = Provincial Laboratory for Public Health
 NML = National Microbiology Laboratory

Refer to the current case definition in the Alberta Health and Wellness Lyme Disease Public Health Notifiable Disease Management Guidelines available at: <http://www.health.alberta.ca/professionals/notifiable-diseases-guide.html>

References:

- (1) Ogden NH, Lindsay RL, Morshed M, Sockett PN, Artsob H. The emergence of Lyme disease in Canada. *CMAJ* 2009;180(12):1221-1224
- (2) Margos G, Vollmer SA, Cornet M, Garnier M, Fingerle V, Wilske B, Bormane A, Vitorino L, Collares-Pereira M, Drancourt M, Kurtenbach K. A new *Borrelia* species defined by multilocus sequence analysis of housekeeping genes. *Appl Environ Microbiol* 2009;75(16):5410-5416
- (3) Centres for Disease Control and Prevention. Recommendations for Test Performance and Interpretation from the Second National Conference on Serologic Diagnosis of Lyme disease *MMWR Morb Mortal Wkly Rep* 1995;44(31):590-591
- (4) Public Health Agency of Canada, Lyme Disease Fact Sheet <http://www.phac-aspc.gc.ca/id-mi/lyme-fs-eng.php> (accessed 30 June 2010)
- (5) 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
- (6) Aguero-Rosenfeld ME, Nowakowski J, McKenna DF, Carbonaro CA, Wormser GP. Serodiagnosis in early Lyme disease. *J Clin Micro* 1993;31(12):3090-3095
- (7) Glatz M, Golestani M, Kerl H, Mullegger RR. Clinical relevance of different IgG and IgM serum antibody responses to *Borrelia burgdorferi* after antibiotic therapy for erythema migrans: long-term follow-up study of 113 patients. *Arch Dermatol*. 2006;142:862-868
- (8) Craft JE, Grodzicki RL, Steere AC. Antibody response in Lyme disease: evaluation of diagnostic tests. *J Infect Dis* 1984;149(5):789-795
- (9) Aguero-Rosenfeld ME, Nowakowski J, Bittker S, Cooper D, Nadelman RB, Wormser GP. Evolution of the serologic response to *Borrelia burgdorferi* in treated patients with culture-confirmed erythema migrans. *J Clin Micro* 1996;34(1):1-9
- (10) Feder HM Jr., Gerber MA, Luger SW, Ryan RW. Persistence of serum antibodies to *Borrelia burgdorferi* in patients treated for Lyme disease. *J Infect Dis* 1992;15(5):788-793
- (11) Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme borreliosis. *Clin Micro Rev* 2005;18(3):484-509
- (12) Jurca T, Ruzic-Sabljić E, Lotric-Furlan S, Maraspin V, Cimperman J, Picken RN, Strle F. Comparison of peripheral and central biopsy sites for the isolation of *Borrelia burgdorferi* sensu lato from erythema migrans skin lesions. *Clin Infect Dis* 1998;27(3):636-8
- (13) Wilske B. Diagnosis of Lyme borreliosis in Europe. *Vector-Borne Zoonotic Dis* 2003;3(4):215-227.
- (14) Berger BW, Johnson RC, Kodner K, Coleman BS. Failure of *Borrelia burgdorferi* to survive in the skin of patients with antibiotic-treated Lyme disease. *J Am Acad Dermatol* 1992;27(1):34-37
- (15) Steere AC, Angelis SM. Therapy for Lyme arthritis: strategies for the treatment of antibiotic-refractory arthritis. *Arthritis Rheum* 2006;54:3079-3086
- (16) Steere AC, Klitz W, Drouin EE, Falk BA, Kwok WW, Nepom GT, Baxter-Lowe LA. Antibiotic-refractory Lyme arthritis is associated with HLA-DR molecules that bind a *Borrelia burgdorferi* peptide. *J Exp Med* 2006;203:961-971
- (17) Wilske B, Fingerle V, Schulte-Spechtel U. Microbiological and serological diagnosis of Lyme borreliosis. *FEMS Immunol Med Microbiol* 2007;49(1):13-21