

APL- Public Health Laboratory (ProvLab) Laboratory Testing for Lyme Disease in Alberta – June 2020

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 infected populations of the blacklegged tick, *Ixodes scapularis*, in parts of southern Manitoba, southern and eastern Ontario, southwestern Quebec, New Brunswick and Nova Scotia, whereas *I. pacificus* is the primary vector in British Columbia (Vancouver Island, the lower mainland and in the Fraser Valley)(1). The Public Health Agency of Canada (PHAC) website provides an updated list of these areas at www.canada.ca/en/public-health/services/diseases/lyme-disease/risk-lyme-disease.html#map. 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 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 (<https://www.alberta.ca/lyme-disease-tick-surveillance.aspx>).

The three genospecies causing Lyme disease are *Borrelia burgdorferi*, *afzelii* and *garinii* (collectively referred to as *B. burgdorferi sensu lato*). While all three genospecies are found in Europe and Asia, only *B. 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* (2).

There are other species that can present with Lyme-like borreliosis: *B. bavariensis* and *B. spielmanii*, and to a lesser extent *B. lusitaniae* and *B. valaisiana* in Europe; *B. bissetii* in the USA (3).

In 2011 and 2016, *B. miyamotoi* and *B. mayonii*, respectively, were shown to cause infections in humans and were transmissible by the same tick vector as Lyme disease. *B. miyamotoi* has a global distribution similar to Lyme disease whereas *B. mayonii* is a variant of *B. burgdorferi* with a more limited geographic distribution in the upper Midwest US. The spectrum of illness of both these organisms is similar to a relapsing fever presentation. While *B. mayonii* can be detected by the C6 Lyme enzyme immunoassay and likely the VlsE1/pepC10 assay, *B. miyamotoi* requires specific testing (4,5).

Testing for Lyme disease:

Both serologic and molecular assays can detect the three genospecies (*B. burgdorferi*, *afzelii* & *garinii*) of LD. For polymerase chain reaction (e.g., PCR testing), the clinical indications and sample type are very restricted [see PCR testing (Table 1a inset in Table 1)] and the Microbiologist-on-Call must be contacted prior to sample collection.

Antibody Screening:

Antibody detection and confirmation follows a two-tiered approach in keeping with the recommendations of 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 (6,7,8). Therefore, only samples with positive and equivocal (indeterminate) results are referred to the National Microbiology Laboratory (NML) for confirmatory testing and genospecies identification by the Western Blot or immunoblot assay.

Results from “Lyme Specialty laboratories” often result in an erroneous diagnosis due to the application of interpretive criteria that are less stringent than those of PHAC, CDC and other accredited agencies, together with the use of non-approved assays (9).

Change in LD serology assay: In March 2020, the VlsE1/pepC10 assay replaced the Lyme C6 enzyme immunoassay, which was discontinued and had been used to test for both IgM and IgG antibodies to *B. burgdorferi*, *afzelii* and *garinii*, since March 23, 2012.

The replacement assay detect antibodies to two immunodominant regions expressed by *B. burgdorferi*, namely the VlsE (variable major protein [Vmp]-like sequence expressed) which comprises of alternating variable (VR1-VR6) and invariant (IR1 to IR6) regions (23). The C6 assay detected antibodies to the IR6 invariant region. The second immunodominant region, OspC, is expressed by this spirochete during the transmission route from the tick-vector to the host. The pepC10 antigen is a conserved component of OspC and improves the sensitivity of this assay. Validation studies performed at the National Microbiology and ProVLab show that the performance of these two assays (C6 vs VlsE1/pepC10) is comparable for all three of the *B. burgdorferi* genospecies.

Importance of Travel history:

B. afzelii and *B. garinii*, are the two genospecies solely endemic to Europe, whereas *B. burgdorferi* occurs in both Europe and North America. As confirmatory testing is lab-ordered and based upon travel history given on the requisition, therefore:-

- If travel is within Canada and the USA or none is provided, confirmatory testing only for North American LD (*B. burgdorferi*) will be requested
- If travel to Europe is given, confirmatory testing for *B. afzelii*, *B. garinii* and *B. burgdorferi* will be requested

Note: In early LD infections with B.afzelii and B.garinii serologic cross-reactivity to B.burgdorferi is also limited in the confirmatory assays and samples may test falsely negative.

Table 1: Laboratory Tests for Lyme disease

| Test Name | Test Performance/Indication | Antibody Response |
|---|---|--|
| Lyme VlsE1/pepC10 IgM/IgG Enzyme immunoassay (EIA/ELISA) for serum [Replaced C6 EIA (in May 2020) that had been introduced March 23, 2012] | <ul style="list-style-type: none"> • Screen for LD antibody at ProvLab in suspected cases • Detects all three genospecies of <i>B. burgdorferi</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 (10). • 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 (11). |
| Testing referred to or only available from National Microbiology Laboratory (NML) | | |
| Borrelia IgM Line Blot (Previously 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 • Primarily detects antibody to <i>B. burgdorferi</i>, although IgM antibody to <i>B. afzelii</i> and <i>B. garinii</i> may be detected as well • Turnaround time is approximately 21 days from receipt at NML | <ul style="list-style-type: none"> • IgM antibodies usually decline to undetectable levels after four to six months (12). However, in some patients a longstanding IgM response is detectable despite effective treatment or historic asymptomatic exposure (13). 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 immunoblot (11). |

| 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>B. burgdorferi</i> • In the later stages of untreated infections with <i>B.afzelli</i> or <i>B.garinii</i>, cross-reactivity in this assay can occur to give a positive result • Turnaround time is approximately 21 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 late manifestations of Lyme disease (12,13). • Initiation of antibiotic treatment prior to testing may result in decreased antibody production which will affect the interpretation of the Western Blot (11). • Once IgG antibodies have developed, they can remain detectable for prolonged periods despite adequate treatment (14). • In the absence of travel information only the <i>B. burgdorferi</i> immunoblot assay will be performed |
| <i>Borrelia garinii</i> & <i>B. afzelli</i> 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 requested if travel history outside of N. America is provided • Mainly detects IgG antibody to <i>B. garinii</i> and <i>B. afzelli</i> • An IgM-specific Western Blot to either genospecies is not available • Turnaround time is approximately 21 days from receipt at NML | <ul style="list-style-type: none"> • Travel history is <u>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>None or very limited serologic cross-reactivity between <i>B. burgdorferi</i> and either <i>B. afzelli</i> and <i>B.garinii</i></u>, by the Western blot assay, especially in the early stages of infection. |
| Lyme Disease IgG antibody in CSF | <ul style="list-style-type: none"> • Only performed at NML in strongly suspected 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. |

| 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 (15,17).</p> <table border="1" data-bbox="432 589 1148 1084"> <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 (15). 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 (16). 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 other two genospecies (17). Effective treatment also results in loss of viability to culture the organism from the skin despite the presence of the rash (18), 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. (19,20). 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 | | | | | | | | | | | | | | | |
| 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 | | | | | | | | | | | | | | | |
| 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 (15). | | | | | | | | | | | | | | | |
| 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 (21). | | | | | | | | | | | | | | | |

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

| Lyme VlsE1/pepC10 IgM/IgG antibody EIA | IgM Immunoblot ¹ | 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 are NOT referred to NML for confirmatory testing. |
| 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. 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. May be suggestive of a recent infection with European LD genospecies (<i>B. afzelii</i> or <i>B. garinii</i>). |
| 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 (6,22). |
| POSITIVE/ EQUIVOCAL | NEGATIVE | POSITIVE | Past or treated Lyme disease infection | <ul style="list-style-type: none"> Asymptomatic infection can occur in up to 15% of exposures |
| 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, late, 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. |

¹The Lyme VlsE1/pepC10 screening EIA detects all three genospecies (*B. burgdorferi*, *afzelii* & *garinii*) causing Lyme disease

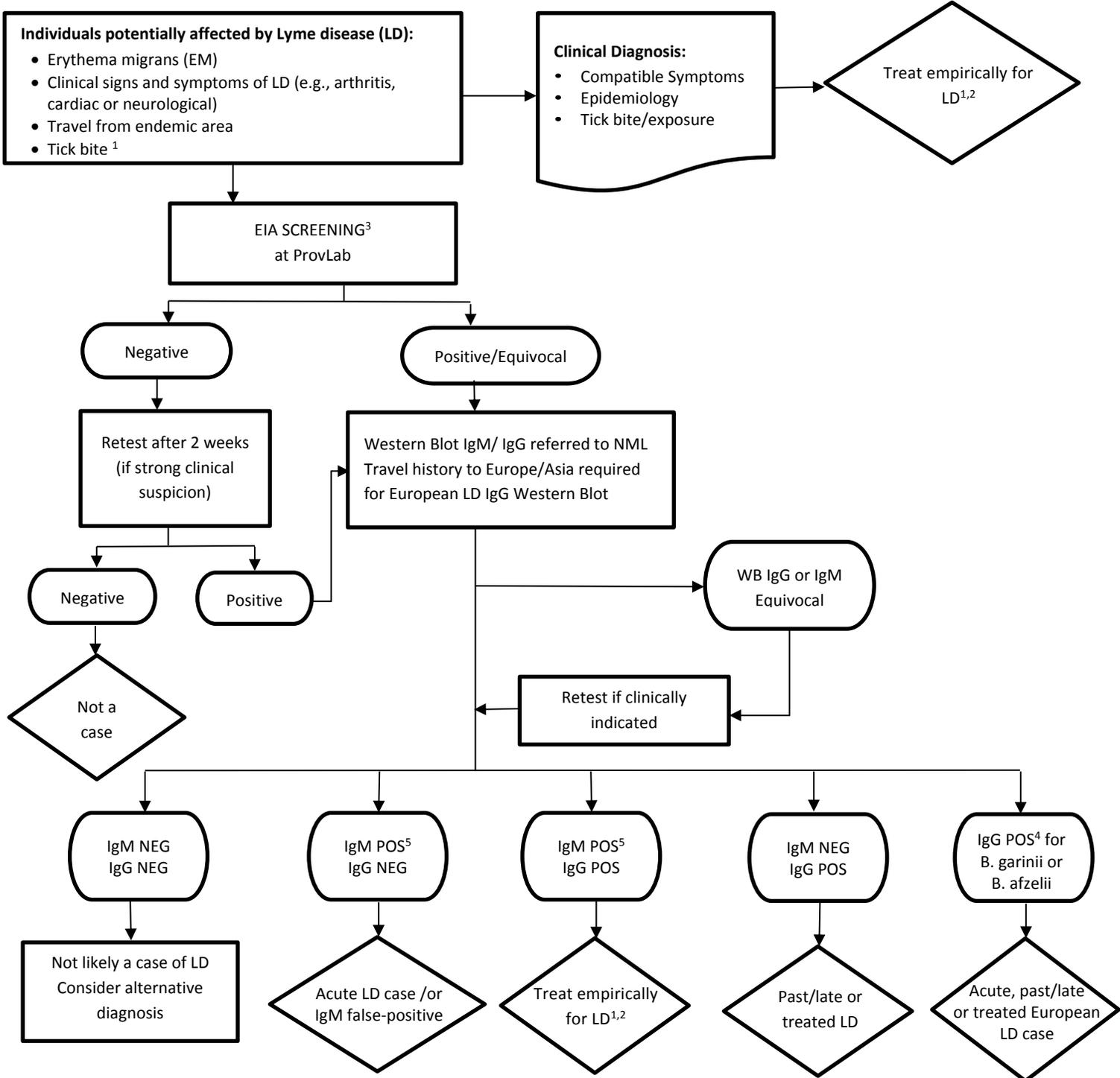
² While the IgM Western/immuno Blot may detect all three genospecies of LD, the IgG Western Blot is specific for *B. burgdorferi* (*sensu stricto*), *B. garinii* and *B. afzelii* where indicated.

³ EQUIVOCAL = INDETERMINATE and POSITIVE = REACTIVE

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Laboratory Testing for Lyme disease and Interpretation, Alberta



¹If the tick is available send to ProvLab for speciation, which can take up to 2 weeks. Ticks identified as *Ixodes spp* are sent to NML for *B.burgdorferi* PCR testing which can take up to 4 weeks. Detection of this organism in the tick does not mean the patient will become infected.

²In consultation with an infectious diseases physician.

³The Lyme VLse1/pepC10 C6 screening EIA detects all three genospecies causing Lyme disease (*B.burgdorferi*, *B.afzelii*, *B.garinii*).

⁴The current IgM immunoblot /Line Blot for *Borrelia spp* may detect IgM antibody to all three genospecies of LD. However staging should be based upon the clinical and epidemiological history as IgM antibody can persist for many months after infection.

⁵IgM antibody positive result <4 weeks after onset of compatible symptoms and in the absence of IgG is suggestive of an acute infection whereas an IgM positive result >4 weeks after onset of compatible symptoms may be a false-positive. An IgM positive result after 4 weeks without a positive IgG result, in the absence of treatment, is strongly suggestive of a false positive finding.