

LABORATORY DIAGNOSIS OF LYME DISEASE: FACT SHEET

- *Borrelia burgdorferi* is a gram-negative loosely coiled spiral bacterium commonly known as Lyme spirochete

LYME IN BC

- The Lyme spirochetes remains very rare in ticks in BC
- Tick sampling in British Columbia takes advantage of ticks removed from people and also sampling from the wild (tested with the sensitive PCR techniques)
- *Borrelia burgdorferi* (the Lyme bacterium) is found in only 1 in 200 ticks in southern BC
 - This rate has been constant between 1996 and 2014
- This rate is 50-100 times lower than in central and eastern North America
- The lower rate may be explained by:
 - Different species of black-legged tick in Western Canada
 - Different forest ecosystem with far less deciduous forest cover
 - Differences caused by the vertebrate hosts
- Global warming does mean that disease range can change
 - This is why the BCCDC keep ticks under surveillance in BC

DIRECT METHODS FOR DETECTION OF BORRELIA BURGDORFERI

- The main direct test modalities used are culture and PCR
- CULTURE
 - Not a routinely available diagnostic method
 - Relatively low sensitivity, long incubation, and the requirement of special media and expertise
 - Cultures need to be kept for 8 to 12 weeks before being considered negative
 - Early and early disseminated infection has a sensitivity of around 40% (Liveris et al. 2011)
 - Rarely cultured from the blood of patients with Lyme disease with later manifestations
 - There are serious concerns with a new serum culture assay that claims a high positivity rate available privately (Johnson et al. 2014)
- POLYMERASE CHAIN REACTION
 - Sensitivity of PCR is similar to culture (Picken et al. 1997)
 - Positive PCR does not necessarily mean an infection is active (Li et al. 2011)
 - Generally used to confirm the presence of borrelia in biopsy material or body fluid

INDIRECT METHODS

- Detect immune response to the organism: Antibody-based assays
- During the acute rash, as many as 50% of patients will be seronegative (Aguero-Rosenfeld et al. 1996)
- In patients with symptoms of more than 1- to 2-months duration, essentially every patient is seropositive (Wormser et al. 2006)
- Some studies performed in the 1980s suggested that early but incomplete treatment with antibiotics might permanently abrogate the antibody response (Dattwyler et al. 1988)
 - Subsequent work showed this T-cell assay to be quite nonspecific, rendering this conclusion incorrect (Dressler et al. 1991)
 - Some have incorrectly interpreted these early studies as indicating that simply ingesting antibiotics would render a patient seronegative (Halperin 2015)
- High incidence of false positives in areas with low prevalence of disease (e.g., BC)
- To improve specificity:
 - A 2-tier approach is used (Marques 2015)
 - The first step uses a highly sensitive ELISA as a screening test
 - If the test is negative, there is no further testing
 - If the test is borderline or positive, then a Western blot is used to provide specificity
 - The sample is retested using separate immunoglobulin IgM and IgG Western blots
 - Results are interpreted using standardized criteria
 - It is important to understand that Western blot criteria were not selected based on the uniqueness of any *Borrelia* epitopes but rather on statistical analyses
 - At least 2 of 3 signature bands are needed for a positive IgM WB, and 5 of 10 signature bands for a positive IgG WB
 - Western blot criteria were developed in individuals with positive or borderline ELISAs
 - Interpretation in patients with negative ELISAs is quite problematic (Halperin 2015)
 - The IgM WB results are used only for disease of less than 4 weeks' duration
 - IgM tests are inherently quite cross-reactive, so false positives are commonplace
 - Patients with disease of more than 1-month or 2-month duration should be IgG seropositive, so only IgG blots provide reliable information. Any IgM findings in this setting should be considered,

at best, uninterpretable, and more correctly as spurious (Halperin 2015)

- In patients with Lyme arthritis, sensitivity of serologic testing is for all intents and purposes 100%, so diagnosis requires positive serology (Halperin 2015)
- In individuals with Lyme disease of more than a month or two duration, sensitivity of serologic testing is over 95% (despite claims to the contrary by many web sites) (Halperin et al. 2013)
- The use of specialty laboratories offering non-validated Lyme diagnostic tests, including unique interpretation of WB results, is discouraged (Fallon et al. 2014)
- Tests using the C6 peptide have significantly improved specificity, and can also be used in patients who acquire the infection in Europe (Branda et al. 2010)
- Fewer than 50% of patients with Erythema Migrans are positive at presentation
 - These patients should receive treatment based on the clinical diagnosis
- Most patients with Lyme arthritis or late neuroborreliosis are positive (Wormser et al. 2013)
- False positives:
 - Positive IgM results for Borrelia can occur in more than 40% of parvovirus B19 infections (Tuuminen et al. 2011)
 - False positives also observed in patients with human granulocytic anaplasmosis, Epstein-Barr virus infections, and patients with autoimmune diseases (Wormser et al. 1997)
 - In addition, false-positive IgM WBs are more common in commercial laboratories (Seriburi et al. 2012)
- 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—i.e., positive serology (including IgM) does not necessarily indicated active infection (Kalish et al. 2001)
- The FDA is concerned with the number of false positives for Lyme Disease from commercial labs
 - See November 16, 2015, report:
 - <http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Reports/UCM472777.pdf>
 - The FDA has generally exercised enforcement discretion towards these tests (i.e., generally not enforced applicable provisions under the FD&C Act and FDA regulations).
 - This will change in the near future
- In British Columbia, Lyme disease prevalence in the tested population is well below 1%, meaning that false-positive diagnoses from an commercial laboratory can exceed true positives by a ratio of at least 50 to 1 (Patrick et al. 2015)

OTHER TESTS

- The clinical usefulness of the following tests has not been established, and these tests should not be used for the diagnosis of Lyme disease (Marques 2015)
- Cell proliferation assays
- Enzyme-Linked ImmunoSpot (ELI-SPOT) assays,
- Cytokine measurements,
- Complement split products, and
- Lymphocyte transformation tests
- Natural killer cell measurements (CD57) are not helpful and do not guide management (Marques et al. 2009)

VEGETATIVE/SPORE/BIOFILM FORMS OF LYME DISEASE

- There is no quality evidence that alternate forms for borrelia play a role in disease inside the human body
- Some of the most widely cited papers speak to what is seen in culture – and serious biofilm scientists point out that the “biofilms” so identified carry none of the ultrastructural features of an actual biofilm – but rather resemble only clumped spirochetes

COINFECTIONS

- In practice, patients with a diagnosis of CLD are often tested for superimposed infections, including:
 - Babesia spp and Anaplasma phagocytophilum (well-described tick-borne pathogens)
 - Bartonella henselae (which is not known to be transmitted by ticks)
- It is important to recognize that, in the context of CLD, a diagnosis of co-infection is most likely spurious and routine testing is not recommended (Halperin 2015)

BCCDC

- The BCCDC has implemented a new serologic test for the routine screening of Lyme disease
 - Zeus VlsE1 pepC10 kit Lyme serology
 - This is an FDA and Health Canada approved test that was validated at BCCDC
- The "VlsE1" component incorporates the C6 peptide, so it is no longer necessary to request a C6 peptide EIA if there is any indication that a Borrelia infection may have been acquired outside of North America (i.e., Europe)

- Its main advantages are:
 - Increased sensitivity for early cases of Lyme disease compared to standard testing
 - The ability to detect non-North American *Borrelia*
 - It appears to be more sensitive for later stage neuro/carditis/arthritis stage Lyme disease

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 Microbiol*. 1996;34(1):1–9.

Branda JA, Aguero-Rosenfeld ME, Ferraro MJ, 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;50:20–6.

Dattwyler RJ, Volkman DJ, Luft BJ, Halperin JJ, Thomas J, Golightly MG. Seronegative Lyme disease. Dissociation of specific T- and B-lymphocyte responses to *Borrelia burgdorferi*. *N Engl J Med*. 1988; 319(22):1441–1446.

Dressler F, Yoshinari NH, Steere AC. The T-cell proliferative assay in the diagnosis of Lyme disease [see comments]. *Ann Intern Med*. 1991; 115(7):533–539.

Fallon BA, Pavlicova M, Coffino SW, et al. A comparison of Lyme disease serologic test results from four laboratories in patients with persistent symptoms after antibiotic treatment. *Clin Infect Dis* 2014;59(12):1705–10.

Halperin JJ. Chronic Lyme disease: misconceptions and challenges for patient management. *Infection and Drug Resistance* 2015;8: 119–128.

Halperin JJ, Baker P, Wormser GP. Common misconceptions about Lyme disease. *Am J Med*. 2013;126(3):264. e261–e267.

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;52:721–4.

Kalish RA, McHugh G, Granquist J, 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;33:780–5.

Lantos PM, Chronic Lyme Disease. *Infect Dis Clin N Am* 29 (2015) 325–340.

Li X, McHugh GA, Damle N, et al. Burden and viability of *Borrelia burgdorferi* in skin and joints of patients with erythema migrans or Lyme arthritis. *Arthritis Rheum* 2011;63:2238–47.

Liveris D, Schwartz I, Bittker S, et al. Improving the yield of blood cultures from patients with early Lyme disease. *J Clin Microbiol* 2011;49:2166–8.

Marques AR. Laboratory Diagnosis of Lyme Disease: Advances and Challenges. *Infect Dis Clin N Am* 29 (2015) 295–307.

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;16:1249–50.

Patrick DM, Miller RR, Gardy JL, et al. Lyme Disease Diagnosed by Alternative Methods: A Phenotype Similar to That of Chronic Fatigue Syndrome. *Clin Infect Dis.* (2015) 61 (7): 1084-1091.

Picken MM, Picken RN, Han D, 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;50:186–93.

Seriburi V, Ndukwe N, Chang Z, et al. High frequency of false positive IgM immunoblots for *Borrelia burgdorferi* in clinical practice. *Clin Microbiol Infect* 2012;18:1236–40.

Tuuminen T, Hedman K, Soderlund-Venermo M, et al. 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;18:167–72.

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;43:1089–1134.

Wormser GP, Schriefer M, Aguero-Rosenfeld ME, 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.

Wormser GP, Horowitz HW, Nowakowski J, et al. Positive Lyme disease serology in patients with clinical and laboratory evidence of human granulocytic ehrlichiosis. *Am J Clin Pathol* 1997;107:142–7.