Ixodes scapularis, Fighting Back, a Clinician's Guide to Lyme Disease Testing in Manitoba: A Critical Appraisal of Current and Proposed Testing Methods

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ABSTRACT

Background: Lyme disease, a tick borne illness caused by *Borrelia burgdorferi*, is an increasingly prevalent infectious disease in Canada. In 2009, there were 128 cases of Lyme disease reported across Canada. In 2015, there were over 700 cases, 35 of those cases in Manitoba. Due to rising temperatures the geographic range of *I. scapularis*, the primary vector of Lyme disease in Manitoba, is enlarging. This will most likely cause an increase in incidence of human infection. As incidence increases it is important to have a full understanding of Lyme disease presentation, testing and treatment. Much of the current research of Lyme disease focuses on testing. Testing for Lyme disease provides useful assistance in diagnosis, however weaknesses exist including insensitivity to early disease and inability to differentiate from active infection or treated infection. This paper will provide a quantitative critical appraisal of current and proposed research for Lyme disease testing.

Methods: A comprehensive Pub Med and Google Scholar database search for "Lyme disease" and "testing" in the last 10 years was conducted. Articles met inclusion criteria if they evaluated any of the testing methods recommended by Infectious Disease Society of America or new proposed methods including: ELISA/whole cell sonicate immunosorbent assay, C6, PCR, iPCR, Western blot, and V1sE. Testing methods had to evaluate serum samples collected from patients with known Lyme disease by symptomatology and confirmatory testing. These articles were reviewed for sensitivity and specificity of testing methods.

Results: In early Lyme disease, standard 2 tier testing was 38-40% sensitive. iPCR hybrid antigen was 55% sensitive. First tier testing C6 ELISA was most sensitive at 64.6%.
In early disseminated Lyme disease, standard 2 tier testing sensitivity increased to 80-88%. PCR and culture decreased to 29% sensitivity. 2 tier ELISA algorithm provided 100% sensitivity and single C6 ELISA provided 90% sensitivity.
In late disseminated Lyme disease, standard 2 tier testing was 92-100% sensitive. Single C6 ELISA was 98.2 % sensitive. iPCR was 92% sensitive and 2 tier ELISA was 100% sensitive. Convalescent samples of serum from patients treated with antibiotics continued to have sensitivity of 87-100% in C6 ELISA.
Specificity of standard 2 tier testing, single C6 ELISA, iPCR and 2 tier ELISA testing was similar ranging from 97-100%.
Specialty labs A and B in the U.S. had 37.8% and 42.5% sensitivity with standard 2 tier testing. Specialty lab B sensitivity changed to 70. 3% when using in-house criteria for interpretation of western blot and specificity decreased from 100% to 72.5%.

Conclusion: Lyme disease testing methods continue to be insensitive to early Lyme disease. Newer methods such as iPCR hybrid antigen provided similar sensitivity to early Lyme disease as current recommend methods. Standard 2 tier testing to disseminated Lyme disease is sensitive and specific. Proposed 2 tier ELISA, first tier C6 ELISA and new iPCR hybrid antigen provide similar sensitivity and sensitivity to disseminated Lyme disease. These methods could potentially be used as alternatives to Western blot to avoid inter-laboratory subjectivity.

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INTRODUCTION

Tick borne illnesses

Anthropoda Acarina Ixodida, commonly known as the tick, can cause a number of human infections. Some of these infections are human granulocytic anaplasmosis (HGA), babesiosis, relapsing fever, Colorado tick fever, Powassan disease, Rocky Mountain Spotted fever (RMSF), rickettsiosis, and tularemia[1]. There are a number of subspecies of Ixodida, but only 2 are responsible for the most common tick borne illness in North America, Lyme disease (LD)[2]. *Ixodes scapularis* and *Ixodes pacificus* are the primary vectors of Lyme disease[3]. The causative bacteria of Lyme disease is *Borrelia burgdorferi*, a spirochete carried by these ticks[4]. *I. pacificus* populations have mainly been reported in southern British Columbia[3]. *I. scapularis* is a vector of Lyme disease in Manitoba, Ontario, Quebec, New Brunswick, Nova Scotia, and Newfoundland and Labrador[3]. *I. scapularis* can also be the vector of *Anaplasma phagocytophilum* and *Babesia microti*[2]. These are the microorganisms that cause HGA and babesiosis[2].

Epidemiology: Canada Wide

The incidence of Lyme cannot really be known until 2009, when it became a notifiable disease. However, a notifiable disease can only be reliable if clinicians are adamant about reporting suspected or confirmed cases. In a US study, under reporting of Lyme is a significant problem. There are about 30,000 cases of Lyme reported to Center for Disease Control (CDC) but an analysis revealed that there were more than 300,000 actual cases Lyme[5].

In 2009, there were 128 reported cases of Lyme in Canada. In 2015, there were 707 cases reported to the Canadian Notifiable Disease Surveillance System[6]. If we apply a factor of 10 to this number, it could be possible that the actual cases of Lyme are closer to over 7,000. Sufficed

to say, Lyme is under reported in North America and the province of Manitoba may be facing the same issue.

Epidemiology: Manitoba Wide

In 2003, one of population of *I. scapularis* was known to be in the southern corner of Manitoba (MB)[3]. Currently, according to surveillance of Lyme disease by the Government of Canada, high risk regions of Manitoba include: West side of Lake of the Woods, Pembina escarpment (including Pembina Valley Provincial Park), St. Malo region, Vita/Arbakka region (including the Roseau River), Beaudry Provincial Park, Assiniboine River and areas next to the Agassiz and Sandilands provincial forests. Lower risk regions also include: parts of southern Manitoba along the border with the United States (from south of Brandon to Lake of the Woods), and some areas around Winnipeg[6]. A surveillance map of these risk regions can be found on http://www.gov.mb.ca/health/publichealth/cdc/tickborne/surveillance.html.

Total confirmed and reported cases of Lyme in 2009 were 11. The number of cases of reported Lyme continued to increase until 2015, when the number slightly fell from 46 (2014) to 35[7]. HGA and babesiosis are also carried by *I. scapularis*. They became reportable in 2015. The first and only reported case of babesiosis was in 2013 in a young boy. In 2016, there have been no reported cases to Public Health Manitoba. There are six known populations of black-legged ticks in Manitoba that carry both *B. burgdorferi* and *B. microti*[8]. However, due to the low incidence of these infections, the main focus of this paper will be only Lyme disease.

Expansion of *I. scapularis*

Due to warmer temps, the geographic range of *I. scapularis* is enlarging[9]. This is an important factor to recognize in Manitoba. In 1997, there was only one known population of *I. scapularis* in Long point, Ontario. By 2007, it has increased to 13 known populations[9]. Nymphs,

which are in the second stage of tick life cycle, are carried on migratory birds that then feed on deer and mice[9]. Temperature and migration of animal hosts are the 2 most important factors that influence tick populations[3]. In a study released by the same author, temperature remains the most influential reason for expansion of *I. scapularis* over other factors such as animal host, habitat or rainfall[10]. Risk maps have been developed up to 2080 to determine expansion of *I. scapularis*. There is moderate risk that *I. scapularis* will be widespread in Manitoba by 2050[9]. In a slower risk scenario, there is moderate risk that they will cover all of southern Manitoba by 2050[9]. This could represent a notable increase in incidence of Lyme disease. It will be necessary to understand Lyme disease as a clinician due to this likely possibility. If temperature averages continue to warm, so too will the incidence of this disease and the health problems associated with it. Health officials in MB should be aware of a highly probable increase of Lyme infection.

PATHOLOGY

Pathology of *B. burgdorferi*

Borrelia burgdorferi is a spirochete bacteria carried on *I. scapularis* and *I. pacificus*. As mentioned before, *I. scapularis* is the major carrier in Manitoba. *I. scapularis* is referred to as the blacklegged tick or the deer tick. The blacklegged tick goes through 4 life stages: egg, larvae, nymph and adult[11]. A tick must feed on blood to grow into the next life cycle. *B. burgdorferi* can be transmitted in any of these stages after they hatch from egg. Each life cycle can last up to one year, while the complete life cycle lasts 3 years[7]. Larvae of blacklegged ticks are as small as 1mm in length and are more light in color. Nymphs are slightly larger at up to 3mm in length and are also very light in color. Adult deer ticks can range in size from 2-5mm. Females are larger and are red and black in color. Males are smaller and are brown in color[7].

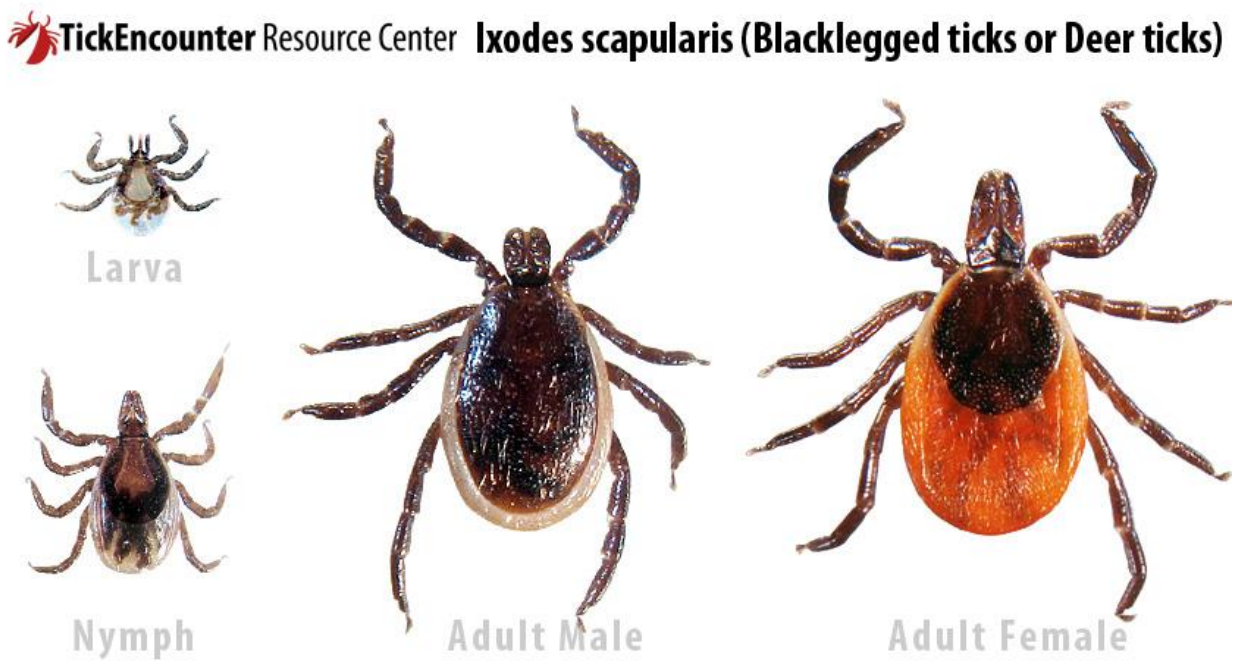

Figure 1: Life stages of *I. scapularis.* http://www.tickencounter.org/tick_identification/deer_tick

Deer ticks can feed on multiple animal hosts, such as mice, birds and humans. Ticks introduce infection with a bite into its host dermis. They insert a feeding tube that has barbs that keep them in place while feeding. Ticks can also release their saliva as an analgesic to prevent the host from feeling their attachment and feeding[11]. They must be attached 36 to 48 hours before the bacteria can be transmitted to local tissue[11]. The earlier the tick is removed, the less likely infection will spread. Spirochetes, spiral and motile bacteria of *B. burgdorferi,* multiply at the site and cause an extending red area, usually with a clear centre. After an initial replication at the site of the bite, the spirochetes spread throughout the body and cause many types of secondary infections[12].

The pathology of how *B. burgdorferi* causes infection is not completely understood. After a primary immune response, some spirochetes are killed but some are able to escape. In secondary response, about 2-4 weeks later, antibodies to *B. burgdorferi* are produced. However, *B. burgdorferi* has a surface protein called V1sE that can code for multiple different surface anti-

gens. Due to this protein, bacteria with an alternate V1sE surface antigen can escape host antibodies[12]. This is how *B. burgdorferi* can continue on to infect other host tissues such as cardiac tissue, nerves, and joints.

Early Localized Lyme Disease (Stage 1)

Early Lyme disease is classically recognized as erythema migrans (EM). This can present as early as 3 days and up to 3 weeks after a tick bite[13]. It can also be accompanied by a fever, headache, stiff neck, myalgia, or arthralagia[13]. EM can be described as a bulls-eye rash: an outer circular red rash with an inner central clearing around a small red circular centre (Figure 2). In up to 80% of cases, EM will be the primary manifestation of Lyme disease[3]. It expands from a small red patch up to 30 cm. To be defined as EM, the diameter must be > 5cm, as smaller than this can be confused with a hypersensitivity reaction to a tick bite. Some may present as a circular erythema without clearing, or the central area may look necrotic or vascular. Other EM may present with multiple rings[14]. EM may persist for a few days up to 4 weeks and may disappear without treatment[13]. It is important to remember that in the other 20% of cases, patients do not remember or did not have EM[13]. This makes it more difficult to make a diagnosis of Lyme when they present with symptoms of disseminated Lyme disease.

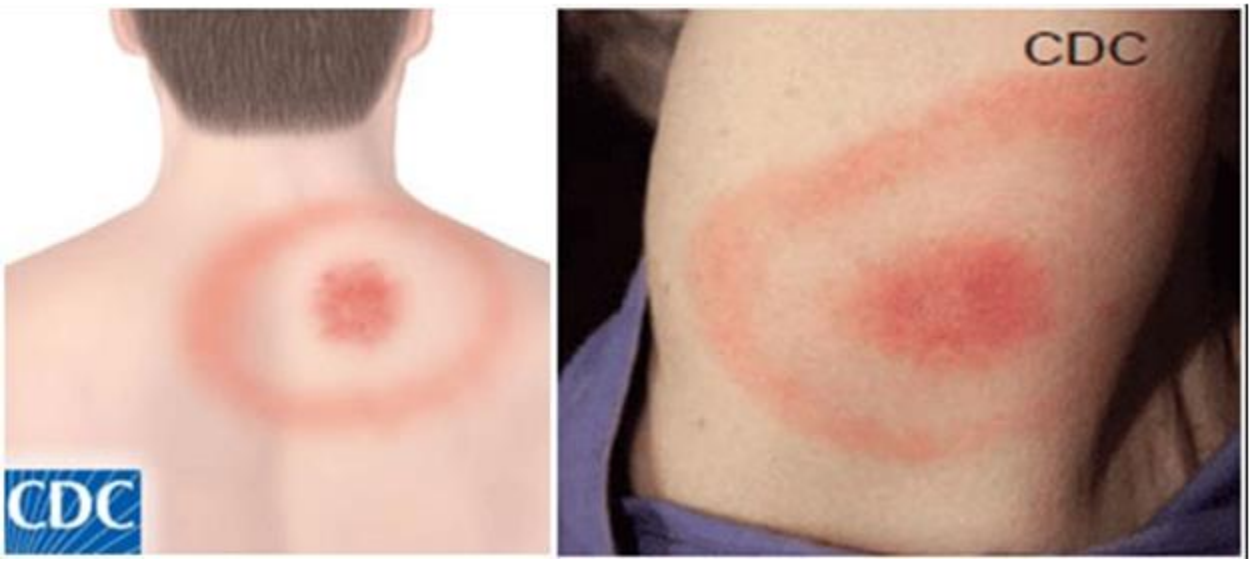

Figure 2: Classic EM of Lyme disease. http://www.cdc.gov/lyme/signs_symptoms/index.html

Testing of early Lyme disease is not currently recommended by Infectious Disease Society of America (IDSA)[2]. Lyme disease testing relies on detecting antibodies to *B. burgdorferi*. Since primary Lyme infection has not yet resulted in antibody generation, testing is not sensitive enough to detect any antibodies in early disease[2]. In most cases, serology will likely be negative for 2-4 weeks[4]. Early localized Lyme disease is a clinical diagnosis that requires the presence of EM and history of residence or visit to an endemic area. However, according to Public Health Agency of Canada, this would be registered as a probable case of Lyme without laboratory evidence of Lyme. Though early Lyme disease testing is insensitive, a confirmed case of Lyme requires clinical evidence of disease and laboratory confirmation of Lyme disease with DNA PCR or standard 2 tier ELISA and Western Blot[15].

These guidelines are for reporting Lyme disease, but clinicians should use their own judgement if Lyme disease is likely without positive laboratory data in early localized Lyme disease and provide the treatment necessary. Treatment of early disease will reduce the chance of

disseminated infection. Laboratory data may not provide a reliable means of confirming Lyme in this stage.

Early Disseminated Lyme disease (Stage 2)

Early disseminated Lyme disease may present several weeks after initial infection. It often occurs in the absence of antibiotic treatment for localized Lyme infection[14]. *B. burgdorferi* is able to spread via blood to multiple organ sites. Secondary lesions of EM can represent dissemination. As well, more rare skin manifestations such as malar rash, diffuse erythema or urticaria may occur[14]. In up to 60% of cases, bacteria can migrate to joints and cause arthralgias[13]. Neurological manifestations can present in up to 15% of patients[13]. There are many early neurologic manifestations of Lyme. Patients can present with cranial neuropathy such as Bell's palsy or facial palsy that can be uni or bilateral. Disseminated Lyme can cause lymphocytic meningitis, encephalomyelitis, radiculoneuritis[2].

B. burgdorferi can also disseminate into the cardiac system. This is a rare finding in about 4-8% of patients[14]. Some patients may present with atrioventricular block with or without myopericarditis. Carditis may also be a nonspecific finding in disseminated disease[2].

Due to the possibility that many of these findings are nonspecific to Lyme, the IDSA guidelines recommend that all patients suspected of Lyme be tested before diagnosis. Early disseminated Lyme is not a clinical diagnosis. A thorough history and physical should be taken to assess contact in an endemic area and to look for EM[2]. If the patient has Lyme disease, most of them will have positive standard 2 tier testing. If clinical suspicion is high and testing is negative, it is important to collect a convalescent specimen about 2-4 weeks after the first specimen[2].

Late Disseminated Lyme disease (Stage 3)

Late Lyme disease is a persistent infection found in about 10% of patients with untreated EM. Many will present with inflammatory arthritis most often affecting the large weight bearing joints such as the knees[14]. If the joint is aspirated, the fluid will have an elevated leukocyte count of 25,000/mm^3 with polymorphonuclear cells predominating[14].

There is also evidence of late disseminated encephalopathy, encephalomyelitis and peripheral neuropathy[2]. Patients should have a history of visit or residence in an endemic area and presence of EM. However, not all patients will have known the presence of EM. In these patients, clinical judgement should be used for suspicion of Lyme disease as the cause of their symptoms.

As with early disseminated Lyme disease, standard 2 tier testing is recommended for all patients with these manifestations and history and clinical manifestations consistent with Lyme disease. Most of these patients will have seropositive testing[2].

Post treatment Lyme disease

Post treatment Lyme disease can be described generally as chronic subjective symptoms after recommended antibiotic treatment regiments with history of manifestations of Lyme disease[2]. Post treatment Lyme disease can be divided into 2 categories: post-Lyme disease symptoms and post-Lyme disease syndrome[14]. Post-Lyme disease symptoms are present in 10-20% of patients. They may complain of fatigue, arthralgia, myalgia for several weeks and up to 6 months[14]. There has also been evidence of cranial nerve palsy while a patient is being treated with appropriate antibiotics, however most is benign and will resolve after completed treatment[2]. In rare cases, some patients will present with Lyme meningitis after full antibiotic therapy. It is imperative to re-treat with antibiotics[2].

Post-Lyme disease syndrome (PLDS) is when subjective symptoms of Lyme persist for longer than 6 months. In a study of post-treatment Lyme symptoms in over 100 patients followed for 10 years after Lyme diagnosis, about 10% of patients were qualified as having post-Lyme disease syndrome that persisted up to 10 years[16]. Of these patients, most complained of memory and concentration problems, followed by fatigue and least commonly, joint pain. In these patients, none were considered to be functionally impaired by their symptoms[16].

In these patients, standard 2 tier testing will not provide any helpful information. As mentioned before, testing relies on antibodies to *B. burgdorferi*, since antibodies to an infection can persist long term, seropositive testing doesn't provide any information on active infection. Some studies have used culture of *B. burgdorferi* to determine active infection, but this has not proved to be an effective determinate of active infection[16].

Chronic Lyme disease

There is no official clinical definition accepted for the term Chronic Lyme Disease (CLD). This is a term that can cause much confusion as it can be applied to PLDS (those with history of Lyme infection). However, the term is also applied to patients with subjective, undiagnosed complaints that have never had history of Lyme infection[2]. It is likely that CLD is an overlap of both of these terms.

There is much debate on the actual existence of CLD. According to IDSA guidelines, CLD patients commonly have other conditions and most patients that have a seropositive test results, will not have Lyme disease. Furthermore, they may improve transiently with antibiotic treatment due to the anti-inflammatory effects of antibiotics[2]. IDSA does not recommend continued antibiotic treatment for post-Lyme disease symptoms after they have received the recom-

mended treatment regiments. It has not been proven to be useful for patients with subjective symptoms for 6 months after treatment[2].

In contrast to these recommendations, Cameron *et al*., believes that the CLD does exist and further research and treatment should be sought out. They pointed out the faults of trials on CLD patients that the IDSA guidelines use as reference. The trials were unaware of the effectiveness of patients' initial treatment, making it possible patients may have been more effectively treated previous to the studies. The trials also provided conclusions without a large enough sample size to do so[17,18]. Cameron *et al*. also notes that many patients continued to have long term illness averaging 4.7 years, further driving his point that CLD exists[17]. They believe that due to these limitations, more antibiotics trials should be planned for patients with CLD. Cameron backs his claim stating that in a previous study, oral antibiotics for a longer term (3 months) are more effective than placebo in CLD patients[18].

Due to the incomplete evidence on CLD, it is important for clinicians to keep an open mind in the setting of possible CLD. In the U.S., CLD can cost a patient more than $1800 in medical costs and greater than $14,000 in loss of productivity and indirect medical costs[18]. With this information, and the understanding that Lyme disease will possibly become a bigger burden to in Manitoba to health care, it will be necessary to continue to follow research on the subject.

TESTING

Testing Outlines

There were more than 3,350,000 Lyme tests performed in a majority of the laboratories in the United States in 2008[19]. In the United States, the IDSA provides the official outlines for testing of Lyme and the CDC provides the laboratory based guidelines. Some of these recommenda-

tions were mentioned previously. The Association of Medical Microbiology and Infectious Disease Canada stands behind these guidelines so a summary of these guidelines will be provided[6].

As mentioned before, IDSA does not believe testing should be relied on for diagnosis of early localized Lyme disease. This does not mean blood tests should not be taken, but a clinical diagnosis can be made with the presence of an EM > 5cm with or without the history of residence or visit to an endemic area. Blood samples should be tested using the same algorithm for other stages of Lyme disease. A first tier ELISA should be performed. If the results are equivocal, the same serum should be confirmed as positive using IgG or IgM Western Blot (WB).

In the case of early or late manifestations of disseminated Lyme disease, IDSA recommends an ELISA with a follow-up Western Blot IgG or IgM. Note that IgM should not be performed if the patient presents > 1 month after symptoms. IgM antibodies appear earlier in infection but are more likely to give false positives. IgG appear later but are more reliable for positive testing[11].

They provide no recommendations on testing for PLDS or CLD. Patients with previous history of Lyme and appropriate treatment may continue to have positive standard 2 tier testing. If a patient with symptoms of CLD presents and has not had a history of Lyme disease or testing, but has history of residing or visiting an endemic area, standard 2 tier could be performed. Once appropriate treatment is provided, patient's serum testing will no longer be clinically useful.

In Manitoba, Cadham Provincial Laboratory provides testing for Lyme disease. First tier testing is a C6 ELISA for antibodies to Lyme. If seropositive or equivocal, serum samples are sent out to the National Microbiology Laboratory to perform IgG/IgM western blots. Lyme PCR can be done from clinical specimens as well, but the sample will be referred out for testing. Turnaround time for testing is around 6-14 working days[20].

Problems with testing

There continue to be weaknesses in Lyme Disease testing. In the presence of early localized disease, testing may be insensitive and relies almost entirely on clinical history and physical. In the presence of initial disseminated early or late Lyme disease, testing can be sensitive and specific. However, clinicians face patients who are more aware of Lyme testing and insist on sending Lyme testing to specialized Lyme testing laboratories in the U.S., if they are unhappy with the results obtained in Canada. Some of these laboratories have their own in-house criteria for interpretation of Western Blot, which has led to variability due to subjective interpretation of Western blot[21].

Furthermore, in the suspicion of post-Lyme symptoms/syndrome and chronic Lyme disease, testing is not useful in guiding treatment and therapy.

Due to the increased risk of cost burden to our health care system in Manitoba as ticks carrying *B. burgdorferi* are expanding, this research will provide a critical appraisal of current and proposed solutions to weaknesses in Lyme testing. More research, testing and statistics should be gathered by health care officials. Also Lyme should be of more concern to clinicians and placed in a realm of possible reasons for symptoms relating to Lyme with history of residence or visiting an endemic area.

METHODS

A Pub Med database search for "Lyme disease" and "testing" in the last 10 years was conducted. Articles met inclusion criteria if they evaluated any of the testing methods recommended by IDSA as well as other methods that may be useful in diagnosis. These included, but not limited to: ELISA/whole cell sonicate immunosorbent assay, C6, PCR, Western blot, and V1sE. Articles had to evaluate blood samples collected from Lyme disease patients with clinical

evidence of Lyme disease in conjunction with seropositive testing. If the cases were not confirmed but collected from suspected Lyme disease patients, they had to be compared to a standard of testing. Based on this search, only 3 articles were acceptable. A further search on Google scholar was conducted and articles were selected as based on previous outlines. This revealed 3 more articles not found on Pub Med. All articles were reviewed for sensitivity and specificity of testing methods.

RESULTS

SENSITIVITY

EARLY LYME DISEASE WITH EM (STAGE 1):

2 tiered testing: ELISA/EIA + IgM/IgG western blot

Wormser *et al*. tested serum samples collected from patients with a clinical diagnosis of EM as well as a positive PCR or culture as gold standard comparison. They found that standard two tier testing for early LD was 38.3% sensitive in these samples. Molins *et al*. collected serum samples from all stages of LD patients and characterized them to provide a serum panel for further research into LD. The serum samples for early LD were collected from patients with a clinical diagnosis of Lyme disease with EM and when possible, positive culture and/or PCR testing as gold standard comparison (n=39). Molins *et al*. found a similar but slightly higher rate of 40% positive when samples where tested using 2 tiered approach.

Branda *et al* reviewed 2 tier testing but, the results were not significant in early Lyme disease due to a high p-value > .05.

PCR/Culture

According to the outlines for confirmed Lyme disease on the Public Health of Manitoba website, positive PCR or culture for Lyme is acceptable for testing for confirmation of LD. Molins *et al*.

evaluated PCR and culture on the same 39 early Lyme disease serum samples previously mentioned. PCR was sensitive to these serum samples at 62% while culture was 44% sensitive. For a combined PCR and culture sensitivity of 65% to acute Lyme disease[22].

iPCR

Halpern *et al* developed and evaluated a synthetic constructed hybrid antigen different than the current panel of whole individual antigens. Multiple antigens are needed for diagnosis of multiple stages and types of Lyme. They developed a single recombinant antigen referred to as DOC antigen to possibly simplify this process (DOC antigen: a full length Dbpa, PEPC10 peptide, and C6 peptide). Serum samples were from clinically diagnosed stage 1 LD patients that were confirmed by PCR or culture. iPCR using this DOC antigen was 55% sensitive to stage 1 LD samples[23].

C6 ELISA stand alone

Wormser *et al*. evaluated the use of a single tiered C6 ELISA kit compared to 2 tier testing. C6 peptide was 64.6% sensitive to early Lyme disease serum samples. This was tested on 498 patients that had acute multiple or single EM, a majority of them previously culture or PCR positive.

Branda *et al*. evaluated 58 convalescent serum samples from patients with previously confirmed stage 1 LD that were treated with recommended antibiotic course. They found that C6 ELISA is 100% sensitive to convalescent stage 1 Lyme disease.

Wormser *et al*. also tested convalescent serum samples (Lyme treated with antibiotics), from stage 1 LD with positive 2 tier testing as gold standard comparison. The sensitivity of C6 ELISA fell slightly to 87%[25].

ELISA/IFA/EIA stand alone

Hinkley *et al*. found sensitivity of standard first tier testing was 66.9% to localized disease. This sensitivity was extrapolated from multiple articles that had previously evaluated these stand alone tests. Hinckley *et al*. did not provide their own sensitivity performance.

EARLY DISSEMINATED LYME DISEASE WITH CARDITIS, NEUROBORRELIOSIS (STAGE 2):

2 tiered testing: ELISA/EIA + IgM/IgG western blot

Molins *et al*. used 17 serum samples collected from patients with well documented Lyme carditis or neuroborreliosis and a clinical diagnosis of stage 2 LD with supportive laboratory data, positive PCR, culture or 2 tier testing, as a gold standard comparison They found the highest sensitivity to early disseminated LD at 88%. Wormser *et al*. tested 44 sera samples from clinically diagnosed neurologic LD with a positive CSF lymphocytic pleocytosis or WCA ELISA as a gold standard comparison. They found a slightly lower sensitivity of 80%. Branda *et al* evaluated 10 serum samples from patients with clinically diagnosed Lyme carditis or neuritis with supportive PCR, culture or 2 tier testing as gold standard. They found a much lower sensitivity of 40% to their samples of early disseminated Lyme.

PCR/Culture

Sensitivity of PCR and culture to the serum samples from stage 2 LD in Molins *et al*. decreased to 29% in early disseminated Lyme disease[22].

C6 ELISA alone

Sensitivity of C6 ELISA to 44 serum samples from stage 2 LD increased to 90% in early disseminated Lyme disease[25].

EIA algorithm

Branda *et al*. investigated a different algorithm than the standard using 2 immunoassays consecutively to evaluate if Western Blot could be avoided. The EIA algorithm used a whole cell sonicate enzyme immunoassay followed by V1sE C6 peptide enzyme immunoassay. They found the sensitivity to the serum samples from confirmed stage 2 LD using this method to be 100% in early disseminated Lyme[24].

LATE DISSEMINATED LYME DISEASE (STAGE 3):

2 tiered testing: ELISA/EIA + IgM/IgG western blot

Hickley *et al*. found sensitivity of standard 2 tier testing was 87%. They based their data on disseminated Lyme and did not specify if this included early and late disseminated Lyme. Again, they extrapolated this sensitivity from multiple other articles.

Halpern *et al*. provided a sensitivity based on stage 2 and 3 serum samples from patients that were clinically diagnosed and had positive standard 2 tier testing as gold standard. The sensitivity based on these samples was 92%. Molins *et al* and Branda *et al* had a total of 40 (n=29, n=11) patients with late Lyme disease all clinically diagnosed and confirmed by standard 2 tier testing previously. They both found 100% sensitivity in these samples.

Worser *et al* tested 114 serum specimens from clinically diagnosed stage 3 LD with a confirmatory WCS ELISA as gold standard comparison and found a sensitivity of 95.6% to these samples using 2 tiered testing.

C6 ELISA alone

Wormser *et al*. tested the same 114 serum samples from stage 3 LD and sensitivity was 98.2% to a C6 ELISA stand alone.

iPCR

Serum samples (n=24) from patients with clinically diagnosed late Lyme arthritis with previous positive standard 2 tier testing. These sera were tested using iPCR. They found iPCR is 92% sensitive to stage 2/3 LD[23].

EIA Algorithm

In serum samples (n=11) for confirmed stage 3 LD with clinical diagnosis and positive standard 2 tier testing as gold standard, the alternative 2 step EIA algorithm tested by Branda *et al*. was 100% sensitive[24].

POST TREATMENT LYME:

37 serum samples of patients with post-treatment Lyme syndrome and 40 control serum samples were evaluated at multiple labs in the United States: 1 university lab, 1 commercial lab and 2 specialty Lyme disease labs. The 37 serum samples were collected from patients with clinical history of and laboratory data of LD. The university lab was used as a reference for gold standard.

ELISA/EIA + IgM/IgG western blot

Sensitivity at the University lab was 48.6%. The commercial lab sensitivity was slightly lower at 40.5%. The speciality labs performed similarly at 37.8% (Lab A) and 43.2 % (Lab B). When using in-house interpretation of IgG western blot, Lab A sensitivity declined to 37.8%. Lab B sensitivity increased to 70.3% [21].

SPECIFICITY

C6 ELISA alone

Branda *et al*. evaluated the specificity of C6 ELISA, using serum samples collected from 1246 healthy subjects, 66 living in an area that LD is endemic, assuming all patients did not have LD. The specificity of these samples was 94%. They also tested serum samples of 1080 blood donors

living in the Boston area, an area of endemicity. They found specificity to this group was 99.4% for C6 ELISA alone. Furthermore, Branda *et al*. found specificity of 100% in 100 blood donors from New Zealand, a non endemic area.

Wormser *et al*. tested 1329 serum samples from areas endemic to LD and 529 serum samples from non-endemic areas. Specificity in non-endemic area to C6 ELISA alone was 99.2%. Specificity in an endemic area was 98.6%. Wormser *et al*. also tested 366 patients with 14 other diseases such as *H. pylori*, Epstein-Barr virus, Hepatitis, HIV, rheumatoid arthritis. They found C6 ELISA was 99.5% specific finding only 2 sera were positive that were also positive in standard 2 tier testing: a rapid plasma reagin and a hemolyzed sample.

2 tiered testing: ELISA/EIA + IgM/IgG western blot

Specificity of standard 2 tier testing performed similarity in all studies.

Halpern *et al*. tested 36 serum samples from patients with other illnesses such as rheumatoid arthritis, multiple sclerosis, periodontitis as well as 24 healthy serum samples. They found that standard 2 tier specificity was 97%. Specificity was lost due to false positives for look-a-like illnesses (not specified in results).

Branda *et al*. tested the same serum samples mentioned previously and found that specificity was 98% in serum samples from healthy donors. Specificity was 99.4% in healthy serum samples from Boston. Specificity was 100% in healthy serum samples from New Zealand, an area of non-endemicity.

Wormser *et al*. tested serum samples from an endemic area and found 2 tier testing was 99.4% specific. In serum samples from an non-endemic area, specificity was 99.8%. Standard 2 tier testing was 99.2% specific in samples collected from other illnesses. 2 tier testing had one more positive result than C6 ELISA to a *H. pylori* sample.

Specificity in specialty Lab A and B were similar at 100% when using standard 2 tier testing on 40 healthy control serum samples[21].

Fallon *et al.* also evaluated IgM WB alone. The specificity in Lab A using in house criteria was also 100%. The specificity changed to 72.5% in Lab B using in house criteria for IgG WB on the same 40 serum samples from healthy controls.

iPCR

Halpern *et al*. found one false positive using iPCR in serum samples collected from an endemic area (n=36), performing at 98% specificity.

EIA Algorithm

Alternative double EIA algorithm was 98% specific to the serum samples from 1246 healthy patients. In the healthy donors from Boston, specificity was 99.4%. In a non-endemic area, specificity of the double EIA algorithm was 100%[24].

Table 1: Results (Sensitivity and Specificity)

Study	Standard 2 tier testing	PCR/Culture	iPCR	EIA algorithm	C6 ELISA or other first tier ELISA	Summary of Results
Hinckley *et al.* Lyme disease testing by commercial labs in U.S.	LD stage 1: 37% LD stage 2/3: 87%	Not evaluated	Not evaluated	Not evaluated	LD stage 1: 66.9% LD stage 2/3: 93.3%	3,351,732 LD tests/year in major U.S. labs 62% 2 tiered 38% stand alone
Molins *et al*. Characterization of samples for LD serum repository	LD stage 1 (n=39): 40% LD stage 2 (n=17): 88% LD stage 3: (n=29): 100%	LD stage 1: 62% PCR, 44% culture LD stage 2: 29%	Not evaluated	Not evaluated	Not evaluated	Stage 1 LD: PCR/Culture/2 tier testing has low sensitivity Stage 2/3 LD: 2 tiered testing is highly sensitive to disseminated disease
Halpern *et al*. Hybrid iPCR antigen	LD stage 1 (n= 20): 40% LD stage 2/3 (n=12): 92% Overall: 59% Specificity (n=36): 97%	Not evaluated	LD stage 1: 40% LD stage 2/3: 92% Overall: 69% Specificity (n=36): 98%	Not evaluated	Not evaluated	iPCR hybrid antigen provides better sensitivity to standard 2 tier testing in early LD. And provides similar sensitivity in disseminated LD and better sensitivity overall. Specificity is similar in both methods.
Wormser *et al.* Single C6 ELISA compared to 2 tier testing	LD stage 1 (n=403): 38.2% LD stage 2 (n= 44): 80% LD stage 3 (n=114): 95.6% Convalescent LD (n=105): 75% Specificity (n=1329,529, 366): 99.4%, 99.8%, 99.2%	Not evaluated	Not evaluated	Not evaluated	LD stage 1: 64.6% LD stage 2: 90% LD stage 3: 98.2% Convalescent LD: 87.5% Specificity: 99.2%, 98.6%, 99.5%	C6 ELISA as a single test provides better sensitivity to early LD and slightly better sensitivity to disseminated LD compared to standard 2 tier testing. Specificity is similar in both methods.

Branda *et al.* 2 EIA algorithm compared to 2 tier testing	LD stage 1: not significant LD stage 2 (n=10): 40% LD stage 3: (n=18): 100% Specificity (n=1246, 1080, 100): 98%, 99,4%, 100	Not evaluated	Not evaluated	LD stage 1: not significant LD stage 2: 89% LD stage 3: 100% Specificity: 98%, 99.4%, 100%	Specificity: 94%, 99.4%, 100%	2 EIA algorithm provides similar sensitivity and specificity to standard 2 tier. Specificity was similar in all methods.
Fallon *et al.* Comparison of specialty labs in the U.S.	Post-lyme disease syndrome: (n=37) University lab: 48.5% Commercial lab: 40.5% Lab A: 37.8% Lab B: 43.2% Specificity (n=40): 100% in all labs	Not evaluated	Not evaluated	Not evaluated	Lab A: 37.8%[t] Lab B: 70.3%[t] Specificity: Lab A: 100%[t] Lab B: 72.5%[t]	Specialty labs provide similar sensitivity in standard 2 tier testing, but major differences are seen when using in house criteria on IgG WB tests

[t] = IgG WB testing using lab in house criteria

DISCUSSION

There are multiple possible solutions tested across these studies to try to optimize Lyme testing sensitivity. The largest study by Hinckley *et al*., provided over 3, 350,000 samples for evaluation. The sample size was very large and provided a good basis for understanding tests ordered and cost of these tests. A majority of Lyme testing ordered, over 60%, was standard two tier testing. They estimated that these tests cost $492 million, a large cost to the health care system. The problem with this article was that they did not evaluate the performance of these tests. Hinckley *et al*. extrapolated percentages from multiple other articles to provide sensitivity and specificity values.

Most studies provided a good analysis of early localized Lyme disease testing. The C6 ELISA provided the highest sensitivity for early Lyme disease testing with a moderate sample size of 114[25]. However, 64.6% is still low for consistent and reliable use for diagnosis. Sensitivi-

ty is likely lost because some patients will present for testing before antibodies have been produced.

iPCR and PCR provided similar sensitivity to C6 ELISA, albeit slightly lower. PCR to DNA of the spirochetes of *B. burgdorferi* provided sensitivity of 62% in early Lyme disease[22]. Sample size was moderate enough to provide a reliable sensitivity value. Though PCR seems like a solution to testing prior to antibody production, it doesn't provide a sensitivity high enough to be acceptable for reliable testing in early LD. iPCR to a hybrid antigen had a sensitivity of 55% in a very small sample size of 12 serum samples[23]. Their method of creating a hybrid antigen to detect Lyme disease seems like a creative solution to simplify testing. It would allow Western blot to be avoided reducing inter-laboratory subjectivity. However, a larger serum sample should be testing before conclusions can be made. Furthermore, standardization and production of the hybrid antigen would have to be undertaken to provide the antigen to all laboratories. Branda *et al*. also provided an alternative to WB, using a 2 tiered immunoassay approach, but their results were not significant in early localized Lyme.

Sensitivity values increased drastically in testing for early disseminated Lyme disease. This is likely in direct relation to the probability that antibodies to Lyme are now formed. An EIA algorithm proposed by Branda *et al*. provided sensitivity of 100%. Unfortunately, there were too few samples to provide reliable conclusion on a 2 EIA algorithm. First tier C6 ELISA was 90% sensitive to stage 2 Lyme, providing a similar sensitivity to standard 2 tier testing that was 80-88% specific depending on the study.

All tests that were evaluated in late disseminated Lyme disease had high sensitivity values. The sensitivity continued to improve, again likely related to further antibody production. The range was 92-100%, providing acceptable rates for reliable Lyme testing. EIA and iPCR

methods performed well, which proves promising, but further testing should be done. Standard 2 tier testing sensitivity was consistent across all studies. C6 ELISA was greater than 98% sensitive to stage 3 Lyme. First tier C6 ELISA had similar sensitivity to standard 2 tier testing in disseminated Lyme. In Manitoba, this assay is first tier testing.

Though overall sensitivity of standard 2 tier testing is similar for the specialty laboratories, there was a significant change if the lab used in-house criteria to interpret IgG WB. Lab A had decreased sensitivity, but Lab B had increased sensitivity by almost 20%. This sample size is small, 77 control and post treatment Lyme samples. But this still illustrates the variability in interpretation of WB if labs do not abide by specific standardized criteria. However, this literature review reveals that there are realistic solutions that avoid WB without affecting specificity of testing such as a 2 tiered EIA algorithm.

Positive testing post-Lyme disease continues to present a problem. Wormser *et al*. demonstrated that post antibiotic treatment Lyme serum samples will continue to have a high sensitivity of 75%. Fallon *et al*. also tested post-treatment Lyme disease samples and sensitivity continued to present a problem. Though this was not the primary focus of the study, it demonstrates the difficulties with treating PLDS when active infection cannot be ruled out as a cause of symptoms.

Syphilis, fibromyalgia, infectious mononucleosis, multiple sclerosis, rheumatoid arthritis and severe periodontitis can cause a false positive in Lyme testing. Specificity remained high throughout most testing evaluation and performance was very similar, from 97% - 100%. Most specificity was lost due to false positives for look-a-like disease, such as *H. pylori*. One notable difference was specificity loss in one specialty lab using in-house lab criteria for IgG WB[21].

CONCLUSION

Incidence of Lyme disease may seem like a small number, but it is likely under reported and under diagnosed posing a greater risk than currently known. Due to increasing temperatures, expanding territory of deer ticks carrying *B. burgdorferi* will further compound this risk. Increased incidence of Lyme will pose a greater burden on the Manitoba health care system and productivity of those affected. If Lyme disease is diagnosed early and more accurately we could decrease the burden of long term cost of Lyme disease to patients and health care. We can do better to ensure patients don't suffer from Lyme disease unnecessarily for months or maybe even years. We need to encourage and seek out new research to improve the current problems with Lyme testing, while also reminding clinicians that there are limitations to Lyme testing. Understanding that there are limitations to standard 2 tier testing currently recommended is a large part of this. This critical appraisal revealed that though standard 2 tier testing is highly sensitive and specific to disseminated Lyme disease, it lacks the sensitivity to early localized Lyme disease. Also, it lacks the ability to determine an active infection after a patient has been treated with appropriate antibiotics. At localized stage, Lyme diagnosis relies on clinicians to be aware of key symptoms and epidemiology, but is simplest to treat and less likely to cause long term symptoms. Furthermore, clinicians must be aware that if patients send samples to specialty labs in the U.S., they should understand there may be some variability in testing with a higher likelihood of a false-positive test due to WB interpretation. 2 tier EIA algorithm and iPCR to a hybrid antigen should be further researched as an alternative to avoid WB all together.

There are some proposed solutions to limitations of Lyme testing. This literature review revealed some of those. There should be continued research on the subject of testing because Lyme disease will continue to be a problem in the future of Manitoba and Canada.

FUTURE RESEARCH

Antigens to improve serodiagnostic testing for early Lyme disease are currently in research phase on animals. Weiner *et al*. used gene products produced by *B. burgdorferi in vivo* infection to test if these could improve early diagnostic testing. These antigens could be developed into recombinant proteins to use Western blot interpretation criteria. They found that this *in vivo* gene product could be helpful in detection of early Lyme disease[26]. MB and other Canadian provinces could benefit from this research and increase the likelihood of detecting Lyme.

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