**Lyme Disease**

Lyme disease is the most common vector-borne disease in the U.S. and is caused by the spirochete *Borrelia burgdorferi*. The upper Midwest is also an endemic area for other tick borne diseases including ehrlichiosis/anaplasmosis and babesiosis. All three infections are transmitted by Ixodes species ticks, primarily the deer tick.

**Ability of Laboratory Tests to Detect Lyme disease**

Misdiagnosis has been a major problem in Lyme disease because of false positive serologic tests, which include the Lyme screen and Western blot. Misinterpretation of Western blots by laboratories that do not follow the criteria established the Centers for Disease Control and Prevention (CDC) has contributed greatly to misdiagnosis of Lyme disease and patient requests for Western blots. There is no evidence that Lyme disease causes a non-specific syndrome of fatigue/myalgias/ poor concentration/headache, etc., with no objective finding (i.e. so-called chronic Lyme disease). This condition is known by many names but it is not chronic Lyme disease and long term IV antibiotics have not been effective for these symptoms in randomized controlled trials. The validity of the 2006 CDC diagnostic criteria for Lyme disease were reaffirmed this month in a report published by the Institute of Medicine (IOM).

Lyme disease is a clinical diagnosis. Currently available laboratory tests, even the newest state-of-the-art tests, are still not sensitive or specific enough to establish the diagnosis. There are many false positives. Laboratory tests can support the clinical diagnosis of Lyme disease but should never be the primary basis for making diagnostic or treatment decisions.

**Problems with lab testing for Lyme disease**

1. A slow antibody response early in the disease (false negatives).
2. Crossreacting antibodies due to endogenous flora such as gram negative enteric rods and oral spirochetes, other infections, or autoimmune disorders (false positives).
3. Very low numbers of organisms for detection by DNA methods (false negatives).

**Detection of Lyme Antibodies**

Serology is the method of choice for Lyme disease testing. Official recommendations from the Centers for Disease Control and Prevention (CDC) are that clinicians use a 2-step procedure when ordering antibody tests for Lyme disease. This procedure starts with a sensitive screening test. Positive screens are followed by Western blot. It is important to remember that the Western Blot is not specific for Lyme disease. Performing a Western blot only when the antibody screen is positive adds specificity to the test.

**Two-Tier Testing for Lyme disease**

**Tier 1: Polyvalent IgG and IgM Lyme antibody screen**

At Allina Medical Laboratories, testing for Lyme disease starts with a sensitive chemiluminescent immunoassay that detects any IgG or IgM antibodies to *B. burgdorferi* proteins (Osp C and VlsE). This FDA-approved assay is more sensitive than currently available ELISA (enzyme-linked immunosorbent assay) methods and has fewer problems with cross reactions and false negatives. Based on studies in patients with culture proven Lyme disease and healthy controls from endemic areas, the reported overall sensitivity of the test is 73% (compared to 41% for ELISA) and the specificity is 95%. 

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**Best Practices in Laboratory Testing**

July 2010
Lauren Anthony, MD, Medical Director
Tier 2: Western blot

When the antibody screen is positive or equivocal, a Western blot should be performed. This will be done automatically when Lyme Antibody Screen with Reflex (659/LYR) is ordered at Allina. The Western blot is another form of antibody test and determines antibody binding to specific proteins. Although there are no proteins that specifically diagnose Lyme disease, the number of proteins recognized in the Western blot is correlated with the diagnosis.

Follow up of a negative Lyme Antibody Screen

In a patient with recent tick exposure or features of early Lyme disease, the screen should be repeated in 2-4 weeks because the antibody response develops slowly. Testing for other tick borne illnesses should also be considered:

Table 1: Tests for other tick-borne diseases endemic to the upper Midwest

<table>
<thead>
<tr>
<th>Human Granulocytic Anaplasmosis (HGA) and Human Monocytic Ehrlichiosis (HME)</th>
<th>Ehrlichia smear (also detects Anaplasma)</th>
<th>Performed at Allina. Same day turnaround.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Erhlichia/Anaplasma PCR (detects and differentiates both organisms)</td>
<td>Sendout test. 1-3 day turnaround.</td>
</tr>
<tr>
<td>Babesiosis</td>
<td>Ehrlichia/Anaplasma antibody</td>
<td>Sendout test. 1-3 day turnaround.</td>
</tr>
<tr>
<td></td>
<td>Malaria/Parasite smear</td>
<td>Performed at Allina. Same day turnaround.</td>
</tr>
<tr>
<td></td>
<td>Babesia PCR</td>
<td>Sendout test. 1-4 day turnaround.</td>
</tr>
<tr>
<td></td>
<td>Babesia antibody</td>
<td>Sendout test. 1-6 day turnaround.</td>
</tr>
</tbody>
</table>

Utility of Western Blot testing in Lyme disease

When the Lyme antibody screen is positive or equivocal, the Western blot is useful to help characterize the antibodies present but is not specific for Lyme disease. In 2009, 61% percent of positive antibody screens performed at Allina Medical Laboratories were also positive by Western blot.

Table 2: Lyme Testing at Allina Medical Laboratories in 2009

<table>
<thead>
<tr>
<th>Total number of Lyme Antibody Screens Performed in 2009 (Detects IgG and/or IgM)</th>
<th>9,075</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of screens with positive or equivocal results</td>
<td>428 (4.72%)</td>
</tr>
<tr>
<td>Number of positive/equivocal screens with a positive Western Blot (IgG and/or IgM)</td>
<td>261 (61% of positive screens)</td>
</tr>
</tbody>
</table>

Misdiagnosis of Lyme disease

Many laypersons, and some healthcare providers, have the erroneous belief that chronic non-specific symptoms alone (e.g. fatigue or arthralgia) may be manifestations of Lyme disease. Allina Medical Laboratories periodically receives requests for Western blot testing in patients who have a negative Lyme antibody screen or for primary screening. Often this is due to a specific patient request, as there are many internet websites with anecdotal reports of chronic Lyme disease detected only by Western blot. Such websites found by Google searching include “Lyme Cryme” and “[Lyme] ELISA and Western Blot: Lies that can kill you?” Misinformation about Western blots in Lyme disease is compounded by a few proprietary laboratories that do not follow CDC criteria for interpreting Western Blots, and report any reaction as positive. These laboratories have contributed greatly to the misdiagnosis of Lyme disease.

Some providers feel that a negative Western blot is the only way to convince some patients that they don’t have Lyme disease. While understandable, acceding to these requests provides no benefit to patients and can lead to more such requests. A resource to help answer questions from patients, titled “Lyme Western Blot: Questions and Answers” is available to help with these requests.
Reinfection with *B. burgdorferi*

Lyme antibodies do not protect against new infections and individuals living in endemic areas may become re-infected. Reported re-infection rates in patients with successfully treated Lyme disease and ongoing exposure to frequent tick bites range from 3% to 28% in endemic areas.

**Antibody screening in patients who have received Lyme vaccine (LYMErix®)**

The *B. burgdorferi* protein used in LYMErix® is Osp A, which is not one of the proteins in the antibody screening test; therefore, LYMErix® vaccination does not cause a positive result in the assay used at Allina Medical Laboratories.

*Borrelia* DNA detection by Polymerase Chain Reaction (PCR)

PCR performs very poorly for detecting *B. burgdorferi* and is not a first line test. Even though PCR can amplify one strand of DNA into millions of copies, the bacterial load in Lyme disease is so low that the specimen volume used for PCR is unlikely to contain even one DNA strand. PCR should only be employed as an adjunctive test in patients who have a positive or equivocal serologic test for antibody to *B. burgdorferi* or are severely immunocompromised. Detection rates in CSF and synovial fluid are better than in blood. A negative PCR result cannot be used to exclude infection.

**Testing of Ticks**

Analysis of ticks to determine whether they are infected is not indicated for diagnostic purposes because it is unclear how the results correlate with the probability of human disease. It should be noted that ticks generally do not transmit *B. burgdorferi* until they have fed for at least 48 hours because the tick must be completely engorged before the Lyme bacteria can migrate to the saliva. Published studies indicate that the transmission rate is 0% for ticks feeding less than 48 hours and 25% for ticks feeding for more than 48 hours.

**References**


LIAISON® *Borrelia burgdorferi* (310870) assay product information, DiaSorin Inc. – Stillwater, MN 55082-0285, USA


CDC guidelines on Lyme testing, [http://www.cdc.gov/mmwr/preview/mmwrhtml/00038469.htm](http://www.cdc.gov/mmwr/preview/mmwrhtml/00038469.htm), accessed 6/25/10.

For questions, comments, or suggestions about this newsletter or other laboratory issues, please contact Lauren Anthony, MD, Medical Director of Allina Medical Laboratories, (612) 262-5013 or Lauren.Anthony@allina.com
Testing for Lyme Disease

Lyme Disease Serology- Polyvalent with Reflex to Western blot
AML Test Code 659/LYR

Positive or Equivocal

Automatic Reflex

Western blot

Negative

Consider testing for Ehrlichia/Anaplasma Babesia (see text)

No further Lyme testing on initial specimen

Consider repeat Lyme screen in 2-4 weeks if clinically indicated.

Positive

Lyme Disease

Consider co-infection with Ehrlichia/Anaplasma

Negative

Consider causes of false positive screening tests (see text)

POSITIVE WESTERN BLOT AND NEUROLOGICAL SYMPTOMS
Order Lyme antibody testing on CSF. Consider PCR testing (low sensitivity, negative results do not exclude neuroborreliosis).

POSITIVE WESTERN BLOT AND KNEE EFFUSION
Perform PCR testing on synovial fluid (relatively good sensitivity but negative results do not exclude Lyme arthritis).