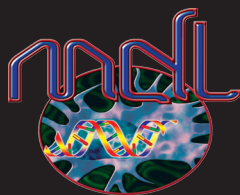


Lyme Disease Testing



Medical Diagnostic Laboratories, L.L.C.

www.mdll.com

Why Use Medical Diagnostic Laboratories for Lyme Disease Testing?

The clinical symptoms of Lyme disease vary among individuals at initial presentation and during the course of an infection, ranging from a relatively benign skin rash to severe arthritic and neurologic symptoms. Diagnosing Lyme disease is no simple task. The clinical symptoms are common to many different disorders. A lot of patients do not present with classic symptoms, which makes a diagnosis based on symptoms alone impossible. Therefore, diagnosis is based on a combination of symptoms and laboratory testing. MDL offers a combination of indirect (Serology) and direct (PCR) testing in order to assist physicians with the diagnosis of Lyme disease. Other laboratory tests are sometimes done to differentiate Lyme disease from other disorders especially Juvenile Rheumatoid Arthritis in children and atypical Rheumatoid Arthritis in adults.

After a person is infected with *Borrelia burgdorferi*, the bacterium that causes most cases of Lyme disease, their immune system will recognize the infection and react by producing antibodies against the bacterium. This is usually a two step process. IgM antibodies are the first wave of attack. They appear within 3-4 weeks after initial infection and hit peak concentrations in about 6-8 weeks. IgM antibodies can persist for several months before they are no longer detectable.

The second phase of response is the production of IgG antibodies. They appear 6-8 weeks after infection and hit their peak concentrations at 4-6 months. Once infected, a person's IgG level may remain detectable for the rest of their life. The spirochetes are tissue loving organisms, therefore, their presence is transient in blood and other body fluids. One of the most important factors in laboratory tests for Lyme disease is timing the collection of the samples. If you obtain the sample too early or the patient doesn't have a strong enough immune response, you may get a false negative test result.

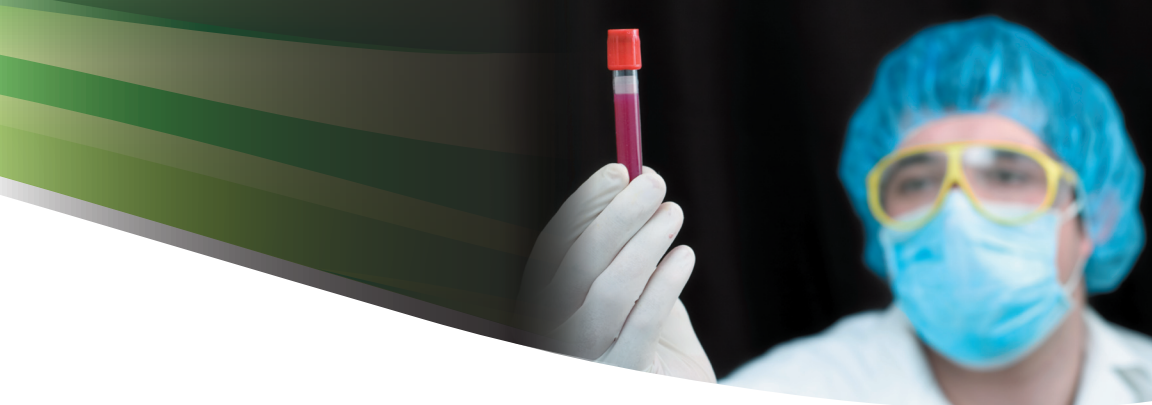
Lyme disease is a vector-borne disease which requires a tick to facilitate the transmission of the disease from the vector (tick) to the patient. The *Ixodes* tick contains additional pathogens that can be cotransmitted with Lyme disease. Other co-infections are babesiosis, Ehrlichiosis, and bartonellosis, which share similar symptoms but different therapy. Therefore, it is paramount to test and identify the vectorborne disease in order to direct therapy.

Other Strains of *Borrelia* *Borrelia garinii* and *Borrelia afzelii*

When *Borrelia burgdorferi*, the bacterium that cause Lyme disease, was originally described, it was believed to be the only species responsible for Lyme borreliosis. However, in the last ten years, different species of *Borrelia* like *B. garinii*, and *B. afzelii* were implicated in clinical cases of Lyme borreliosis. Neurological symptoms seem to be the most common manifestation in western Europe and *B. garinii* is most frequently associated with these cases.

Borrelia garinii is one of two major strains found in Europe. It usually causes Lyme disease symptoms of the neurological kind, such as extreme back and leg pains, meningitis and partial facial paralysis.

There has been considerable speculation concerning possible differences in the ecology of the genospecies and most data suggest that *B. afzelii* is associated with rodents and *B. garinii* with birds. However, recently *B. garinii* has also been identified in tissues taken from rodents and at least one strain of *B. garinii* (serotype 4), rarely found in ticks but associated with neuroborreliosis, appears to be specific for rodents.



Borrelia lonestari

In the southeastern states, a clinical condition similar to Lyme disease, termed southern tick-associated rash illness (STARI), has been described in humans in the southeastern region of the United States associated with the bite of *Amblyomma americanum* ticks. Moreover, a new spirochete, *B. lonestari*, was described from *A. americanum*. Individuals affected with STARI, commonly develop a localized expanding circular skin rash (erythema migrans) at the site of the tick bite similar to that seen with classic Lyme disease. A mild illness characterized by generalized fatigue, headache, stiff neck, and occasionally fever and other constitutional signs accompanies the rash. The disease appears to respond to antibiotic treatment.

STARI, which is also referred to as "Masters' disease" in recognition of the physician who first described its clinical presentation, has been attributed to infection with an as-yet-uncultivated spirochete tentatively referred to as *Borellia lonestari*. Cases consistent with this clinical presentation have been reported from several southeastern and south central states, including Missouri, Maryland, Georgia, South Carolina, and North Carolina. The majority of patients with STARI do not have laboratory evidence of infection with *B. burgdorferi* sensu stricto.

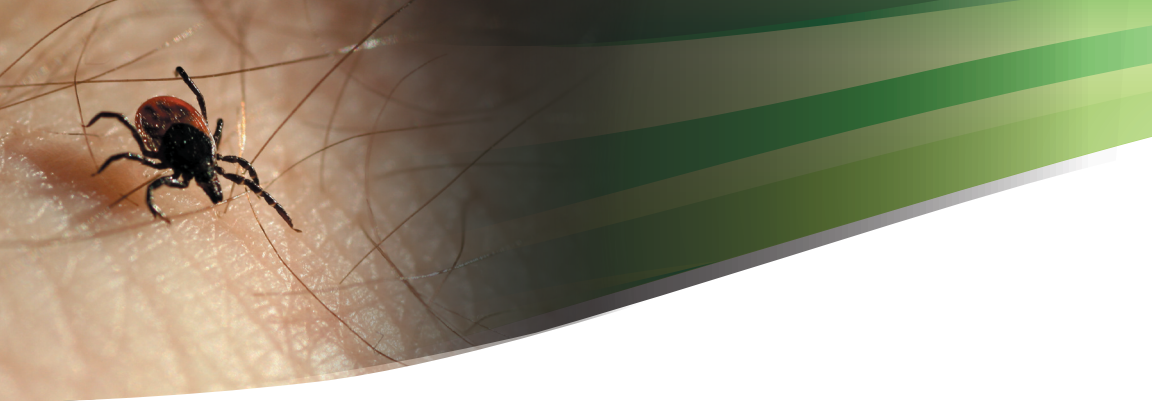
Types of Laboratory Testing

Antibody tests are indirect because they measure exposure to the bacterium. A positive test is a function of the host immunological response to a foreign antigen, i.e. *B. burgdorferi*. Direct tests are those that detect the entire bacterium (usually by culture or staining techniques) or parts of the bacterium such as cell wall proteins, carbohydrates, intracellular proteins, or nucleic acids such as DNA or RNA. Both indirect (Serology) and direct tests (PCR) should be routinely used for the accurate, sensitive, and specific laboratory detection of Lyme disease. Because of the complex nature of the disease, no single test is adequate for all clinical situations.

MDL suggests that three initial tests be used to detect both acute (IgM) and later (IgG) specific antibodies to *B. burgdorferi*.

Available Direct (PCR) Test for Lyme disease:

- ***B. burgdorferi* by Real-Time PCR (#305)**
 - A positive PCR test result is proof of the presence of the Lyme disease-causing bacterium in the specimen
- ***Borrelia afzelii* by Real-Time PCR (#424)**
 - European strain
- ***Borrelia garinii* by Real-Time PCR (#425)**
 - European strain
- ***Borrelia lonestari* by Real-Time PCR (#430)**
 - Southern strain



Available Indirect (Serology) Tests for Lyme disease:

- **Lyme disease IgG/IgM by ELISA (#427)**
 - Highly specific to reduce false positive results
- **Lyme disease Western blot (IgG/IgM) (#313)**
 - Performed when Lyme ELISA has returned an equivocal or positive result
 - Provides both IgM/IgG Western blot results
 - Detect and analyze IgM/IgG Western blot results
 - Increased specificity reduces cross-reactivity from other spirochetes
- **Lyme disease C6 Peptide (C6LPA) by ELISA (#417)**
 - Detects both IgM and IgG antibodies in patients with Lyme disease as well as in patients who have received the vaccine
 - Capable of detecting antibodies to all US & European strains of *B. burgdorferi*
 - Highly specific and more sensitive for diagnosing Lyme disease
 - No interference with antibodies due to vaccination

Specimen Types for DNA PCR Testing:

- Whole blood
- Urine
- Synovial fluid
- Ticks
- Serum
- Cerebrospinal fluid (CSF)
- Biopsy

Collection Procedures

1. Obtain a laboratory order in writing from your physician.
2. **Whole blood** – draw in yellow-top, ACD- Solution A vacutainer tube.
3. **Serum** – draw in a SST-red top vacutainer tube, mix by inversion, incubate at room temperature for 15 minutes, centrifuge, pour off and send on an ice pack.
4. **Urine** – collect in a sterile urine container and send on an ice pack or collect in a **UroSwab®** transport vial and send at room temperature.
5. **CSF, Synovial Fluid or Biopsy** – must be collected by a physician and placed in a sterile redtop vacutainer tube or biopsy vial.
6. **Ticks** – the head must be intact; place tick in a zip lock bag, a clean bottle or any dry container. Tick may be dead or alive. DO NOT remove with alcohol or a hot match.
7. Send specimen(s) to Medical Diagnostic Laboratories by overnight delivery with physician's order, insurance information and credit card information.
8. Results will be faxed to the ordering physician.

Other Tick-borne diseases:

Many Lyme disease patients have multiple co-infections. In addition to the Lyme spirochete, *Borrelia burgdorferi*, a subset of Lyme patients are also infected with other tick-borne pathogens like mycoplasma, rickettsia and/or protozoa. MDL is capable of detecting other tick-borne disease agents such as *Babesia microti*, *Ehrlichia chaffeensis* (HME) and *Anaplasma phagocytophila* (HGE), Rocky Mountain Spotted Fever (RMSF), *Bartonella henselae*, and *Mycoplasma* spp.

Babesia

Babesia is an intra-erythrocytic protozoa that is transmitted by the same *Ixodes* tick implicated in Lyme disease. There are about 99 different species of *Babesia* but it is usually the *Babesia microti* that is implicated in infection in the United States. Symptoms of *Babesia* can often be nonspecific and include fatigue, malaise, chills and headache. Some of the symptoms also mimic malaria. However, most cases of human babesiosis are probably sub-clinical and occur as a self-limiting illness. People who are older or ill with other conditions may experience the symptoms of babesiosis. These may first appear within one month to one year after exposure and include fatigue and loss of appetite. As the infection grows more severe, symptoms may include fever, chills, drenching sweats, muscle aches, jaundice and headache.

Babesiosis occurs principally in the spring, summer and fall in the coastal areas of the United States, especially on the off-shore islands of New York and Massachusetts. Bite cases of babesiosis have been reported in Georgia, California, and Wisconsin, as well as in Europe. Babesiosis is currently considered a health threat throughout the US, as cases of the infection and deaths have occurred in areas where the risk of infection was not believed to exist previously.

Available Tests for Babesia:

- *Babesia microti* by Real-Time PCR (Direct) (#410)
- *Babesia microti* IgG by ELISA (Indirect) (#419)

Bartonella

Patients with a history of Lyme disease who have incomplete resolution of symptoms should be evaluated for *Bartonella* infections. *Bartonella* is an intracellular, gram-negative bacteria that can become chronic.

Bartonellosis is a common name for cat scratch disease which is a flea-borne disease that carries the *Bartonella* bacteria. MDL was the first laboratory to detect the presence of *Bartonella henselae* in the *Ixodes scapularis* ticks.

The disease is generally mild, the symptoms of bartonellosis typically include swollen lymph nodes and fever. Swelling in the lymph nodes (armpit, neckbone and above the collarbone) may persist for months. Headaches, joint pain, and eye redness may occur.

Bartonella may not present in its usual form when additional infections, such as Lyme disease or *Babesia* are present. In addition, typical *Bartonella* lesions are not always seen in patients, therefore, a diagnosis of "fever of unknown origin" should alert a physician to consider *Bartonella*. It is estimated that approximately 2/3 of the patients with *Bartonella* have a fever. Involvement of practically every organ has been reported.

There are a variety of symptoms associated with *Bartonella*, including, but not limited to, fatigue, restlessness, combative behavior, myalgias, malaise, liver and/or spleen involvement, abdominal pain, infectious mononucleosis-like syndrome, granulomatous hepatitis.

Available Direct PCR Tests for Bartonella:

- *Bartonella henselae* by Qualitative PCR (#317)

Ehrlichia

Ehrlichia is an obligate intracellular gram negative bacteria of the Rickettsiaceae family. It is transmitted by the *Ixodes* tick, the same vector implicated in Lyme disease and babesiosis. It is more prevalent in the months of May through July. There are several different species of *Ehrlichia* but the species suspected in human Ehrlichiosis is *E. chaffeensis*. The symptoms are non-specific and flu-like. They include fever, myalgias, malaise, headache and chills. The absence of a rash can help to distinguish it clinically from Lyme disease and Rocky Mountain Spotted Fever.

Available Direct PCR Tests for Ehrlichia:

- *Ehrlichia chaffeensis* (HME), *Anaplasma phagocytophila* (HGE) by Real-Time PCR (#411)

Mycoplasma

A subset of Lyme disease patients also have mycoplasmal infections (*Mycoplasma fermentans*, *Mycoplasma hominis*, *Mycoplasma pneumoniae* or *Mycoplasma penetrans*.) In some cases multiple mycoplasmal infections can be present in Lyme disease. The presence of mycoplasmal infections complicates the diagnosis and treatment of Lyme disease, and some of the generalized signs and symptoms found in Lyme disease-positive patients are also found in mycoplasma-positive patients.

Like the *Borrelia* spirochete, the mycoplasmas are found at intracellular locations in various tissues and are only rarely found in the blood. This can make detection difficult because of the cyclical appearance of *Borrelia* and various mycoplasmas in the patient's blood cells. Mycoplasmal infections can independently cause many of the signs and symptoms found in Lyme disease, and they may exacerbate the clinical signs and symptoms and complicate treatment of the condition.

Available Direct PCR Tests for Mycoplasma:

- *Mycoplasma fermentans* by Qualitative PCR (#332)
- *Mycoplasma hominis* by Real-Time PCR (#130)
- *Mycoplasma penetrans* by Qualitative PCR (#335)
- *Mycoplasma pneumoniae* by Qualitative PCR (#336)

Rocky Mountain Spotted Fever

Rocky Mountain Spotted Fever (RMSF) is the most severe and most frequently reported rickettsial illness in the United States. The disease is caused by *Rickettsia rickettsii*, a species of bacteria that is spread to humans by *Ixodid* (hard) ticks. Initial signs and symptoms of the disease include sudden onset of fever, headache, and muscle pain, followed by development of a rash. The disease can be difficult to diagnose in the early stages, and without prompt and appropriate treatment it can be fatal.

Available Direct PCR Tests for Rocky Mountain Spotted Fever (RMSF):

- *Rickettsia rickettsii* (RMSF) by Qualitative PCR (#416)



Treatment of Lyme Disease

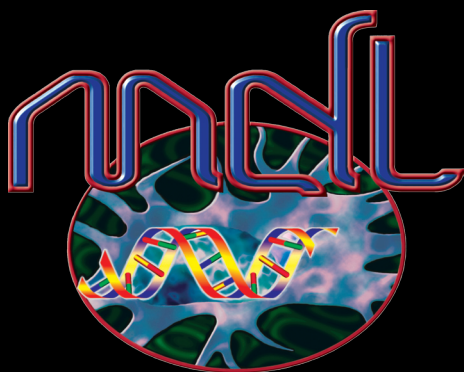
Patients with *Borrelia* infections or combinations of *Borrelia* and *Mycoplasma* and/or *Babesia* and/or *Ehrlichia* infections can do well on combinations of antibiotics plus nutritional and nutritional support. Experts agree that Lyme disease is much easier to treat in the earlier phases, but the co-infections can be difficult to treat, especially if the disease is in the late chronic persistent stage.

The most common recommendations for the treatment of Lyme disease *Borrelia* infections involve antibiotics that can effectively suppress early localized or early-disseminated Lyme disease *Borrelia*.

The clinical diagnosis of tick-borne diseases is difficult, especially in the early stages of infection. Inconsistent and nonspecific clinical presentation of symptoms and unreliable traditional laboratory testing complicate the physician's ability to accurately diagnose and treat patients. The combination of serology and PCR testing offered by Medical Diagnostic Laboratories provides the clinician an accurate, rapid, highly sensitive and specific means for early detection.

Preventing Transmission of Tick-borne Pathogens

- Avoid endemic areas during the months of May through September
- Wear appropriate clothing:
 - long pants tucked under socks
 - long sleeved shirt to cover the arms
 - light colored clothing to enhance easy detection of crawling ticks
 - wear a hat in densely wooded areas
- Use of tick repellent:
 - Dimethyl phthalate
 - Diethyl toluamide (DEET) *use sparingly on the skin surfaces of young children
- Daily examination and removal of ticks



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