CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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Lyme Blot, IgG and IgM
- Now Performed at CPAL –

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Affected Tests:

Mnemonics:	Lyme PG	Lyme Blot
Test Name:	Lyme IgG/IgM Screen—Progressive (Includes reflex to Lyme Blot)	Lyme Blot Group (Includes IgG and IgM)
Test Number:	3003082	1757060
Specimen:	1 mL serum, 2-8^{0}C up to 7 days, -20^{0}C or colder up to 6 months	

Effective Date: Testing will begin on February 3, 2014. Specimens received beginning January 30, 2014 will be held for testing on February 3, 2014.

Performed: Monday and Thursday, with additional testing as needed during Lyme season

Reference Range: Negative

Method Change:
CPAL currently performs Lyme IgG/IgM screening with reflexed confirmatory test sent to Quest Diagnostics. To improve service for the members, CPAL now performs Lyme confirmatory testing by the MarDx Lyme IgG and IgM Western Blot method. Results will be provided in the same LIS format as previously provided when performed at Quest Diagnostics, so that there will be no LIS changes needed by the members. Each result will be accompanied by a comment alerting to method change.

Background:
Lyme disease is a multisystem disease caused by a spirochete, *Borrelia burgdorferi*. The disease has been documented in Europe since early this century. It has been recently documented in the United States during an epidemic in 1975 among children in Old Lyme, Connecticut, who demonstrated arthritic symptoms. Steere et al. recognized the disease as a separate clinical entity. Its symptoms may be nonspecific and confused with those of juvenile rheumatoid arthritis, lupus erythematosis, multiple sclerosis, rheumatic fever, Reiter's Syndrome, myocarditis, and viral meningitis.

The organism is transmitted through an arthropod vector from an animal reservoir. *B. burgdorferi* was first isolated from *Ixodes dammini* ticks, which was shown to be the etiologic agent. *Ixodes scapularis* ticks are principally responsible for transmission in the northeastern and mid-Atlantic regions, as well as in Minnesota and Wisconsin. *Ixodes pacificus* transmits *B. burgdorferi* in California coastal and mountain regions.

The animals that may be infected: deer, wild mice, birds, raccoons, horses, dogs, and cats. The ticks are commonly found on vegetation in epidemic areas especially in wooded areas common to the infected animals. The incidence of human infection coincides with the tick season from May through September in most parts of the United States.
Among the many symptoms of early onset of Lyme disease are:

1. A red lesion on or near the site of a tick bite. The lesion is called Erythema Migrans (EM).
2. Arthritic symptoms
3. Low grade "flu like" fever
4. Headaches
5. Dizziness
6. Stiff neck
7. Fatigue and general malaise
8. Muscular aches and pains
9. Abdominal pain
10. Irregular pulse and heart beat

EM develops in up to 60 - 70% of the cases within a few days to weeks following a tick bite. The lesion typically starts at the site of the bite and radiates slowly in a circular pattern. It may reach 5-50 cm in diameter and generally clears centrally within a few weeks. The lesion, unfortunately, cannot be relied upon for the clinical diagnosis of Lyme disease. Additionally, because of the very small size of the nymphal tick (1-2 mm), as many as 80% of tick bites are unrecognized. During this period symptoms of headache, malaise, myalgia, fever, arthralgia, fatigue, and lymphadenopathy are usually present.

In the later stages, symptoms can resemble a variety of different diseases. Neurological, Cardiac and Musculoskeletal Involvement generally are symptoms that may appear from weeks to months following initial infection. This stage is characterized by symptoms of dizziness, weakness and irregular heartbeat, meningitis, inflamed nerve roots in the neck, and facial palsy. Other symptoms include: mood swings, loss of memory, inability to concentrate, poor motor coordination, and somnolence.

Arthritic Symptoms: Generally the large joints are affected with pain and swelling. The arthritic attacks may be recurrent. Isolation of *B. burgdorferi* from skin biopsy, blood, and spinal fluid has been reported and is definitive for establishing *B. burgdorferi* infection. However, these direct cultural methods cannot be practically relied upon for routine laboratory diagnosis of *B. burgdorferi* infection. Overgrowth by the competing microflora, complicated growth medium requirements, and the slow growth rate of the spirochete are all factors influencing the outcome of direct culture.

Serologic methods are the most commonly used for presumptive diagnosis of infection. Both enzyme immunoassay (EIA) and indirect immunofluorescence assays (IFA) have been employed. EIA is considered more sensitive and less subjective than IFA. The use of Western Blot is useful for characterizing the specificity of the antibody response to *B. burgdorferi*. This discrimination is not possible with the IFA or EIA because these procedures measure the total antibody response only. Steere

et al. reported that patients with Lyme disease produce antibodies of the IgM class during the first few weeks after onset of EM and produce antibodies of the IgG class more slowly. Both IgM and IgG titers can remain positive for many months or years. Persons with very early stages of Lyme disease treated with antibiotics may not develop titers or will develop only low antibody levels. Antibody cross reactions in Western blot and other serologic methods have been reported with other pathogenic spirochetes such as *Treponema pallidum*, the causative agent for syphilis, and *T. pertenue*, the causative agent for yaws. The VDRL (Venereal Disease Research Lab) or RPR (Rapid Plasma Reagin) tests may be useful for differentiating treponematosis (the VDRL or RPR are usually positive) from Lyme disease without treponemal exposure (the VDRL and RPR are negative).

Method Principle:
The MarDx *B. burgdorferi* (IgG) Strip Test System is a Western blot technique utilizing antigens of *B. burgdorferi* (Strain B 31) which are separated in the presence of sodium dodecyl sulfate (SDS) by polyacrylamide gel electrophoresis. The resolved protein bands are then transferred by electrophoresis to a nitrocellulose membrane. The membrane is dried, cut into strips, and packaged.

Serum is incubated with individual Marblot *B. burgdorferi* strips. If specific antibodies to individual proteins are present, they will bind to the corresponding *B. burgdorferi* antigen bands. After washing the unbound serum from the strip, the bound *B. burgdorferi* specific antibody is reacted with alkaline phosphatase conjugated anti-human IgG. The strip is then washed to remove the unbound conjugated antibody, and the strip is finally reacted with a precipitating color developing solution which deposits a purple precipitate on antibody reacted antigen bands. Bands are visualized, scored for intensity relative to the 41kD band of the Weakly Reactive Control and recorded.

The immune response to *B. burgdorferi* infection appears to follow a classic response pattern. Serum IgM can be detected in some patients within days after disease onset. At about four weeks after onset, the IgM response has attained maximum serum concentration and complexity of Western blot banding patterns. Serum IgG is detected as early as two weeks after onset. Significant concentrations of antibody and Western blot banding patterns for *B. burgdorferi* can be found years after onset.

Results Interpretation:
Both IgG and IgM Lyme Blots are interpreted according to the number of significant positive bands detected as shown in tables 1 and 2 respectively.

Table 1: Interpretation of Lyme IgG Blot

Criteria for Interpretations	Reported as
Positive: Any 5 of the following 10 bands: 18, 23 ,28 ,30 ,39, 41, 45, 58, 66, or 93kDa	IgG antibodies to significant *B. burgdorferi* proteins detected; presumptive evidence of probable exposure.
Negative: Patterns other than positive	IgG antibodies to less than 5 of the 10 significant *B. burgdorferi* proteins detected; or no IgG antibodies to significant *B. burgdorferi* proteins detected. Additional specimens should be submitted in 2-4 weeks if *B. burgdorferi* exposure has not been ruled out.
Unreadable	The lyme blot is uninterpretable due to the specimen repeatedly exhibiting non specific binding to the strips used in the Lyme Blot assay. Recommend submitting a new sample for Lyme Blot testing within 2 to 4 weeks.

Table 2: Interpretation of Lyme IgM Blot

Criteria for Interpretations	Reported as
Positive: Any 2 of the following 3 bands: 23, 39, 41kDa	IgM antibodies to significant *B. burgdorferi* proteins detected; presumptive evidence of probable exposure.
Negative: Patterns other than positive	IgM antibodies to less than 2 of the 3 significant *B. burgdorferi* proteins detected; or no IgM antibodies to significant *B. burgdorferi* proteins detected. Additional specimens should be submitted in 2-4 weeks if *B. burgdorferi* exposure has not been ruled out.
Unreadable	The lyme blot is uninterpretable due to the specimen repeatedly exhibiting non specific binding to the strips used in the Lyme Blot assay. Recommend submitting a new sample for Lyme Blot testing within 2 to 4 weeks.

Limitations:

1. The MarDx *B. burgdorferi* (IgG) Marblot Strip Test System should only be used to test human serum samples which have been found positive or equivocal using an EIA or IFA test procedure. Western blot should not be performed as a screening procedure.
2. Individuals with POSITIVE Western blot for antibodies to *B. burgdorferi* should be referred for medical evaluation which may include additional testing. The diagnosis of Lyme Disease must include careful clinical evaluation and should not be based only on detection of antibodies to *B. burgdorferi.*
3. If a specimen repeatedly yields unreadable blots and symptoms persist, a fresh specimen should be tested in 2-4 weeks.
4. A negative western blot does not exclude the possibility of infection with *B. burgdorferi.*
5. Sera from individuals with other pathogenic spirochetal diseases such as syphilis, yaws, pinta, leptospirosis, relapsing fever, and periodontal disease may give false positive results. Individuals with connective tissue autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosis, and individuals with anti-nuclear antibody may also give false positive results. Individuals with other bacterial and viral infections such as Rocky Mountain Spotted Fever, Epstein-Barr Virus, and cytomegalovirus may also have antibodies which cross-react with *B. burgdorferi.*
6. The continued presence or absence of antibodies to *B. burgdorferi* cannot be used to determine the success or failure of therapy.
7. A positive *B. burgdorferi* IgG Western Blot result only indicates probable immunologic exposure, however, the presence of an Immunologic response has not been correlated with active infection.
8. When testing specimens from patients during early *B. burgdorferi* infection, 0 to 4 weeks after onset of symptoms, the IgG test is less sensitive. A suitable IgM Western blot test should also be used for early detection of antibodies to *B. burgdorferi*.
9. Studies have demonstrated that antibiotic therapy may or may not affect the seroconversion from IgM to IgG during the course of the disease.

Validation Data:

Within run and between run precision were performed on both positive and negative samples for each assay, with 100% agreement.

Manufacturer's claims for assay performance are as follows:

Sensitivity: (89% w/ 95% CI = 75.4-96.2%) positive % agreement at 1-2 months post exposure.
Specificity: (94.3% w/ 95% CI = 92.4- 95.9%) negative % agreement with known results.

Method comparison studies were performed using frozen aliquots stored at CPAL versus Quest Diagnostics' original results. Any discrepancies were resolved by sending fresh aliquots to Quest for retesting. Results are shown in tables 1 and 2 for IgG and IgM Lyme Blots respectively. There was 100 concordance in results interpretation for both assays.

Table 1: Lyme IgG Blot Patient Comparisons

MarDx Lyme IgG Blot Positive and Negative Concordance			
	# Quest IgG Blot Positive	# Quest IgG Blot Negative	TOTAL
# MarDx IgG Blot Positive	11	0	11
# MarDx IgG Blot Negative	0	10	10
TOTAL	11	10	21
% Agreement	100%	100%	

Table 2: Lyme IgM Blot Patient Comparisons

MarDx Lyme IgM Blot Positive and Negative Concordance			
	# Quest IgM Blot Positive	# Quest IgM Blot Negative	TOTAL
# MarDx IgM Blot Positive	10	0	10
# MarDx IgM Blot Negative	0	10	10
TOTAL	10	10	20
% Agreement	100%	100%	

REFERENCES:

MarDx Lyme IgM Marblot Strip Test System Package Insert (REF#40-5065M) 04/2012

MarDx Lyme IgG Marblot Strip Test System Package Insert (REF#40-5065G) 04/2012