LYME DISEASE SEROLOGY TEST UPDATE
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SUMMARY
Effective May 8, 2013, the Division of Laboratory Medicine will make the following changes regarding the serological diagnosis of Lyme Disease (LD):

1) The LYME ANTIBODY SCREEN, EARLY and
   LYME ANTIBODY SCREEN, LATE reflex panels will be replaced with a single reflex panel, LYME DISEASE SEROLOGY, SERUM. This panel will include the following tests:
   a) Lyme Antibody EIA, always performed;
   b) Lyme IgM & IgG Immunoblots, performed only when the Lyme EIA is positive or equivocal.

2) Requests for immunoblots on Lyme EIA-negative sera will no longer be honored.

(For more information and rationale on these changes see Diagnosis below.)

BACKGROUND
LD, caused by the spirochete bacterium Borrelia burgdorferi, is the most common tick-borne illness in our region. B. burgdorferi is a zoonosis of deer and various small mammals, primarily the white-footed mouse and the chipmunk, spread by the hard-bodied tick Ixodes scapularis (aka deer tick). Humans are incidentally infected by a tick bite, usually the second stage nymph. Because of the tiny size of the nymph, most humans do not recall being bitten, even though the tick will typically stay attached for up to several days.

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Phases of LD may be broken into three groups:
1) The Local Early phase, arising within several days to several weeks after the tick bite. Manifestations typically include mild non-specific flu-like symptoms and the characteristic annular red skin lesion at the site of the tick bite, known as erythema migrans (EM). EM is seen in approximately 80% of cases but is not always of the typical appearance.
2) The Disseminated Early phase begins within several days to several weeks of the primary EM lesion’s appearance. Secondary EM lesions are common, denoting a spread of the organism, and the various constitutional symptoms become progressively worse. Without treatment, frank neurologic and/or cardiac symptoms will develop within about 20% of untreated patients. Other organ systems can also be affected.
3) Late LD develops in untreated patients weeks to years (average six months) after the initial tick bite. Reoccurring, intermittent attacks of arthritis, usually of the large joints and especially the knees, occur in about 60% of patients with late LD. Joint effusions with a leukocytic cytosis of up to 110,000 cells/mL are common. Additionally, chronic neurologic manifestations may rarely occur.

DIAGNOSIS
Serological studies are the cornerstone of LD diagnosis. The CDC recommends that all previously undiagnosed possible LD cases be tested by a two-tier algorithm:
1) All sera are to be initially tested with an anti-\textit{B. burgdorferi} antibody enzyme immunoassay (EIA).
2) All EIA positive or equivocal sera are then confirmed with \textit{B. burgdorferi} antibody immunoblot (IB) assays.
   a) IgM & IgG IBs in first month of infection (i.e., during the EM period), or
   b) IgG only thereafter.

The sensitivity of the CDC two-tier algorithm ranges from 40% in the first weeks of infection to nearly universal seropositivity in untreated Late LD. The IgM IB is necessary to maximize sensitivity early in the infection, but is prone to false positive results; a positive IgM/negative IgG IB result after the first 1 – 2 months of illness is most likely a false positive result. When reviewing LD serology results, it is important to remember two things:
1) LD patients can remain seropositive for months or years, even after successful treatment. Repeat testing when reinfection is suspected is therefore of little clinical utility.
2) There are no published data that support the appearance and disappearance of individual bands on IBs as a tool in diagnosis. It is thus more useful to rely on the interpretation of an IB, and not on the individual bands reported.

The change to a single reflex panel composed of both IgM & IgG IBs was made in order to simplify test selection and reduce delays in diagnosis. \textit{It is important to note that IgM IB data will now always be provided when the EIA is positive or equivocal regardless of the stage of infection. The clinician should therefore be highly cautious of a positive IgM/negative IgG IB result in cases clearly beyond the Local Early phase.}

Please be aware that IBs will no longer be performed on EIA-negative sera. This decision was made in consultation with our infectious disease specialists, is fully endorsed by the Laboratory Compliance
Committee, and follows CDC recommendations. LD IBs, when interpreted using the CDC criteria of ≥2 out of 3 IgM bands and ≥5 out of 10 IgG bands as a positive result, are no more sensitive than modern EIA assays. Furthermore, interpreting LD IBs as positive when fewer bands are present, while possibly correctly identifying additional cases of LD, will also lead to incorrect diagnoses of LD, cause unnecessary antimicrobial treatment, and in some cases lead to a missed true diagnosis. When Local Early LD is suspected and the LD antibody screen test is negative, retesting once in 2-4 weeks is recommended. Beyond the Local Early phase, a negative EIA result confidently rules out LD in the immunocompetent patient.

It is important to remember that serological testing is not 100% accurate. The diagnosis of LD is thus largely a clinical one that requires a thorough history & physical exam by the clinician, as well as careful consideration of the diagnostic study results.

**TEST INFORMATION**

**TEST NAME:** Lyme Disease Serology, Serum

**TEST CODE:** LYMPAN

**SPECIMEN REQUIREMENTS:**
1.0 mL serum (Red Top Tube preferred, Serum Separator Tube acceptable)

**MINIMUM VOLUME:**
0.5 mL serum

**REJECTION CRITERIA:**
Bilirubinemic, hemolyzed, lipemic and turbid sera are unacceptable
CSF is unacceptable

**AVAILABILITY:**
Lyme Antibody Screen (EIA) performed Sunday through Thursday
Lyme Confirmatory Immunoblot performed Monday, Wednesday, Friday during summer and Friday during off season.

**REFERENCE VALUE:**
Negative

**ADDITIONAL INFORMATION:**

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A TEST FOR ANTIBODIES TO PHOSPHATIDYLSERINE PROTHROMBIN COMPLEX IS NOW AVAILABLE AT MARSHFIELD LABS

Michael J. Sanfelippo, MS, MT(ASCP)

Antiphospholipid syndrome (APS) is an autoimmune disorder associated with a high incidence of thrombotic disease and obstetrical complications. This condition is identified by demonstration of the persistent presence of either the lupus anticoagulant or antibodies to cardiolipin or $\beta_2$-glycoprotein I.

Recently, antibodies to the complex of phosphatidylserine and prothrombin (aPS/PT) have been identified in patients with systemic lupus erythematosis (SLE) and well characterized APS. In order to determine if the demonstration of aPS/PT would be helpful in the identification of APS, a study was performed at Marshfield Labs. Specimens submitted for identification of antibodies to cardiolipin and $\beta_2$-glycoprotein I that had normal levels of these antibodies were retested for antibodies to PS/PT. Over 700 specimens were tested. Forty-one (41) specimens (6%) were found to have elevated levels of antibodies to PS/PT. Nineteen of the specimens with elevated antibodies to PS/PT were from patients without accessible medical records. The other 22 specimens were from patients with histories of pulmonary emboli, deep venous thrombosis, neurologic disorders, SLE, and other autoimmune or connective tissue disorders.

While the measurement of aPS/PT will identify patients with antiphospholipid syndrome who have normal levels of antibody to cardiolipin and $\beta_2$-glycoprotein I, the measurement of antibodies to cardiolipin and $\beta_2$-glycoprotein I is still the most efficient way to begin the evaluation for antiphospholipid syndrome. Testing for aPS/PT is best utilized as follow-up testing in patients without demonstrable antibodies to cardiolipin, $\beta_2$-glycoprotein I, or the lupus anticoagulant. When the comprehensive antiphospholipid panel is negative, a comment will be included in the report suggesting consideration of aPS/PT testing.

The test used for the measurement of aPS/PT is FDA cleared and will report both IgG and IgM class antibodies. The reference range for the IgG and IgM class antibodies is 30 units or less. Patients having levels greater than 30 units for either IgG or IgM antibodies have a level of antibody that has been associated with autoimmune disease including the antiphospholipid syndrome.

The test will be performed in the special hematology department one day per week. It became available on April 10, 2013. The preferred specimen for this test is serum.

QUESTIONS
Please contact Dr. Thomas Novicki or Dr. Thomas Fritsche with clinical and interpretive questions regarding this test, or Dr. Joyce Flanagan with technical questions at 800-222-5835.

REFERENCES
For further information, please contact:
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Technical Director of Coagulation Services
Marshfield Labs
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REFERENCES: