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DETECTION OF BABESIOSIS AND AVAILABILITY OF A NEW, HIGHLY SENSITIVE, NUCLEIC ACID AMPLIFICATION TEST (PCR)

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SUMMARY

Beginning May 13, 2013, Marshfield Laboratories will introduce PCR testing for detection of the tick-borne pathogen *Babesia microti* from whole blood specimens.

The lab test code will be BABNAT.
This test will replace the BMPCRSO test.
This test can also be ordered as part of a Tick Panel (TICKP) which includes *Anaplasma, Ehrlichia* and *Babesia microti*.

This test will be offered in addition to traditional blood-smear analysis and serology testing, and will be most appropriate for testing early in the infection cycle when symptoms are acute. The PCR assay offers a high level of sensitivity and specificity, and compliments the other diagnostics although each has strengths and limitations, details of which follow.

BACKGROUND

Babesiosis is a tick-borne malaria-like illness caused by a protozoan of the genus *Babesia*. *Babesia microti* is the species responsible for the vast majority of human cases in the United States [1] and is transmitted by the deer or black-legged tick, *Ixodes scapularis* (see http://en.wikipedia.org/wiki/File:Babesia_life_cycle_human_en.svg). The same species of tick also transmits the causative agents of Lyme

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disease (*Borrelia burgdorferi*) and anaplasmosis (*Anaplasma phagocytophilum*). Dual infections with these agents have been described [2]. Babesiosis may also be transmitted by blood transfusions from an asymptomatic donor [3].

CLINICAL PRESENTATION (From the Marshfield Clinic *Clinical Practice Guideline: Babesiosis* [4,5])

A clinical history of tick exposure is helpful though an actual tick bite is not always documented. The incubation period for babesiosis is usually one to three weeks, but may extend occasionally up to six weeks or up to nine weeks in post-transfusion disease. Symptoms may range from asymptomatic to hemolytic anemia with multi-system organ failure. Most commonly seen are non-specific flu-like symptoms (fever, chills, sweats, myalgias, arthralgias). Splenomegaly, hepatomegaly and jaundice may be found on physical exam. If a rash is noted, the patient may have a co-infection with Lyme disease. Laboratory findings of hemolytic anemia, thrombocytopenia, and conjugated bilirubinemia could indicate babesiosis. These findings should prompt further evaluation for evidence of hemolysis. Less specific laboratory findings of proteinuria, elevated LFT's, elevated creatinine, and elevated BUN may also be found. Severe disease can occur in patients with certain comorbidities (i.e., elderly, asplenia or otherwise immune-compromised) where the infection causes hemolytic anemia, thrombocytopenia, renal failure and even death.

TESTING OPTIONS

Laboratory testing presently includes hematologic, immunoserologic and molecular studies. While molecular results are highly sensitive and specific indicators of disease, they should be correlated with blood smear microscopy and serological results, as required, and with clinical findings.

Hematologic Testing

Traditional blood smear analysis using EDTA-preserved blood samples has been a standard assay for detecting babesiosis, and is a rapid assay available in most clinical laboratories having the capability of identifying this parasite. The assay primarily detects intraerythrocytic

TEST INFORMATION

HOW TO ORDER THIS TEST:
BABESIA MICROTI Nucleic Acid Test, NAT, BLOOD
KEYWORDS: *Babesia microti*

LAB TEST CODE: BABNAT
CLINIC (CLINICAL ORDER MANAGER):
Babesia microti, NAT,
HOSPITAL (CENTRICITY):
Babesia microti, NAT
DOWNTIME: Write-In (Form I)

Or

LAB TEST CODE: TICKP (includes Babesia, Anaplasma, Ehrlichia)
CLINIC (CLINICAL ORDER MANAGER):
TICK BORNE PANEL, NAT
HOSPITAL (CENTRICITY):
TICK BORNE PANEL, NAT
DOWNTIME: Write-In (Form I)

SPECIMEN REQUIREMENTS:
Local – Draw blood in a lavender-top (EDTA) tube(s), and send 5 mL of refrigerated EDTA whole blood. Do not centrifuge.

Outreach - Draw blood in a lavender-top (EDTA) tube(s), and send 5 mL of refrigerated EDTA whole blood. Do not centrifuge.

MINIMUM:
0.2 mL

REJECTION CRITERIA:
Samples drawn in heparin tube or yellow-top (ACD) tube are not acceptable.
Samples frozen or at room temperature are unacceptable.

STORAGE:
Refrigeration.

AVAILABLE:
Test is set up Monday through Friday; analytic time of 1 day.

QUALITATIVE INTERPRETATION:
Positive or Negative

CPT CODES:
BABNAT = 87798
TICKP = 87798 x 2

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trophozoites of *B. microti* and related species in symptomatic patients. The test detects patent parasitemia approximately two weeks from tick exposure and is highly specific, but lacks sensitivity compared with a molecular PCR assay because parasitemia is highly variable.

Serologic Testing

Detection of antibodies specific to *B. microti* may be useful in patients with a high likelihood of disease despite undetectable parasitemia, or to assess prior exposure. Antibodies usually appear shortly after symptoms are apparent and can be expected to remain elevated for months to years; detection of antibodies is not useful for monitoring the effectiveness of therapy. Testing of acute and convalescent specimens (separated by at least two weeks) may be useful to better differentiate recent from distant or past exposure. Recent infection is usually manifested by a single high titer, or a four-fold rise in titer when paired samples are tested. Because of the inherent delay in the appearance of antibodies following exposure, some individuals may test negative during the early stages of infection and give false negative results. Use of the PCR assay can be expected to provide greater sensitivity during the early stages of infection, with the serology results being complementary and demonstrating evidence of infection at later time points when smear and PCR tests may be negative.

Molecular Testing

PCR testing of whole blood is capable of detecting minute quantities of the organism's specific DNA and is frequently positive prior to a detectable serologic response. The PCR used in our laboratory was developed based on a method published by Teal, et al.[6]. The assay includes an additional non-*Babesia* target DNA which serves as an internal control to ensure there are no inhibitors of PCR in the test sample. This PCR methodology demonstrates 100% sensitivity at a concentration of 500 *Babesia* organisms per mL of blood (corresponding to 1 out of 2,000,000 RBC's infected). In contrast, the limit of detection of traditional blood smears is about 250,000 *Babesia* organisms per mL of blood (corresponding to 1 out of 4,000 RBC's infected). To express this another way, the limit of detection for this PCR test is about five hundred times greater than traditional blood smear microscopy.

SAMPLE RESULT INTERPRETATIONS:

Sample Negative NAT Report:

Negative, no DNA matching that of *Babesia microti* was detected. These results do not exclude the presence of the organism or active/recent disease.

Sample Positive NAT Report:

Positive for *Babesia microti* by PCR. Positive results indicate presence of specific DNA from *Babesia microti* and support the diagnosis of babesiosis.

Sample Equivocal NAT Report:

This sample was tested in duplicate for *Babesia microti* by PCR. The amount of *Babesia microti* detected in both runs is much less than that found in most symptomatic patients, but may indicate a very early or resolving infection. Repeat testing is recommended if clinically indicated.

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Sample Indeterminate NAT Report:
This sample was tested in duplicate for *Babesia microti* by PCR. Result is indeterminate due to a PCR inhibitor present in the specimen. Consider repeat specimen if clinically indicated.

All reports will include the following information:
The PCR result should be interpreted in conjunction with other laboratory tests, including chemistry, hematology and serology results, patient history and clinical presentation.

This test was developed and its performance characteristics determined by Marshfield Labs. It has not been cleared or approved by the U.S. Food and Drug Administration(FDA). The FDA has determined that such clearance or approval is not necessary. This test is for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity testing.

QUESTIONS

For questions and additional information, please contact:
Dr. Uphoff, Dr. Novicki or Dr. Fritsche at 1-6700 or 800-222-5835

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