Marshfield Labs™
A division of Marshfield Clinic

# Laboratory News

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## New Nucleic Acid Amplification Test (PCR) for *Anaplasma* and *Ehrlichia* Species

Timothy Uphoff, Ph.D., DABMG, MLS(ASCP)CM; Tara Weiler; and Thomas Fritsche, MD, Ph.D., FCAP, FIDSA

### SUMMARY

Beginning August 1, 2012, Marshfield Labs will introduce PCR testing for detection of the tick-borne pathogens *Anaplasma phagocytophilum, Ehrlichia chaffeensis, Ehrlichia ewingii/canis* and *Ehrlichia muris-like* (EML) from a single whole blood specimen.

The lab test code will be: **ANENAT**
This test will replace the ANPCRSO test.
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.

### BACKGROUND

Anaplasmosis is a tick-borne disease caused by the bacterium *Anaplasma phagocytophilum* and is transmitted by the widespread blacklegged deer or bear tick (*Ixodes scapularis*). Before 2001, the organism was known as *Ehrlichia phagocytophila* and the disease produced as ehrlichiosis. The infection was first recognized in 1993 from several patients in Minnesota and western Wisconsin; at that time the disease was known as human granulocytic ehrlichiosis (HGE) and has since been called human granulocytic anaplasmosis (HGA). The number of anaplasmosis cases reported to the CDC has increased steadily since the disease became reportable, from 348 cases in 2000, to 1006 cases in 2008. The case fatality rate (i.e., the proportion of anaplasmosis patients that reportedly died as a result of infection) has remained low, at less than 1% (CDC, 2006).



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Rarely, related forms of ehrlichiosis caused by *Ehrlichia chaffeensis* (producing human monocytic ehrlichiosis) and *E. ewingii* (producing human granulocytic ehrlichiosis) have been reported in the Midwest. These bacteria are carried by the Lone Star tick (*Amblyomma americanum*), found throughout much of the southeastern and south central United States, but rarely in Wisconsin. Another related form of ehrlichiosis caused by the *Ehrlichia muris-like* (EML) agent was identified in Minnesota and Wisconsin patients in 2009 (Pritt, Sloan, et al. 2011). Since then a few cases have been reported in both states. It appears that *Ixodes scapularis* may also carry this disease agent and transmit it to people.

The signs and symptoms of human anaplasmosis and ehrlichiosis may include:

- Fever
- Headache
- Chills
- Malaise
- Muscle pain
- Nausea
- Confusion
- Conjunctival injection (red eyes)
- Rash (rare with anaplasmosis)

Other symptoms can include vomiting, loss of appetite, weight loss, abdominal pain, cough, diarrhea, aching joints and change in mental status. Laboratory findings that may be helpful in the diagnosis of ehrlichiosis in an acutely febrile patient after tick exposure include leukopenia and/or thrombocytopenia, and elevated serum aminotransferase levels. Although people of any age can get human anaplasmosis or erhlichiosis, the infections appear to be most severe in the aging or immune-compromised populations. Severe complications can include respiratory failure, renal failure and secondary infections.

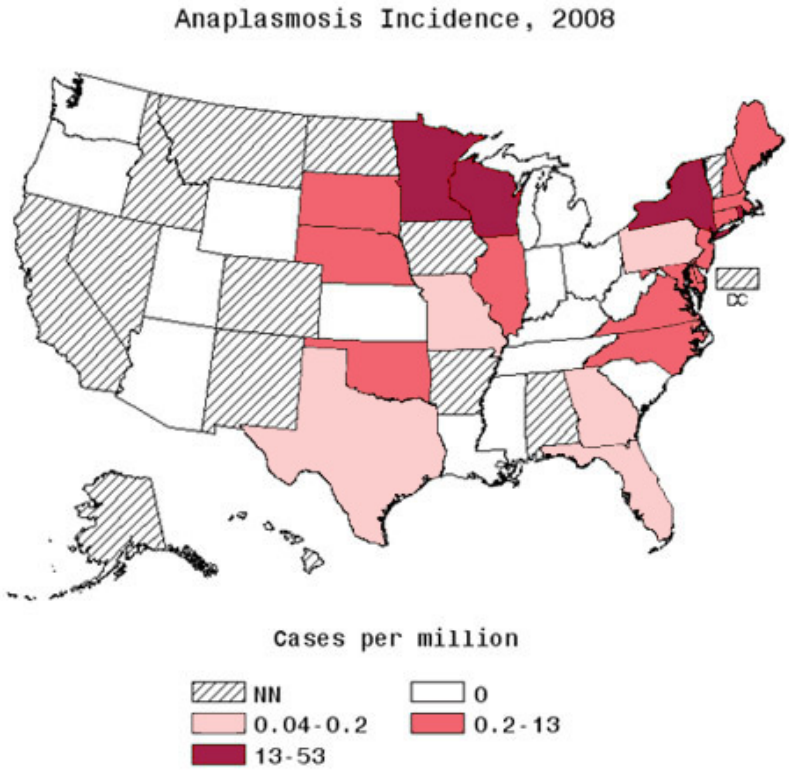


Figure 1. Annual reported incidence (per million population) for anaplasmosis in the United States for 2008. (NN= Not notifiable) (CDC, 2012).

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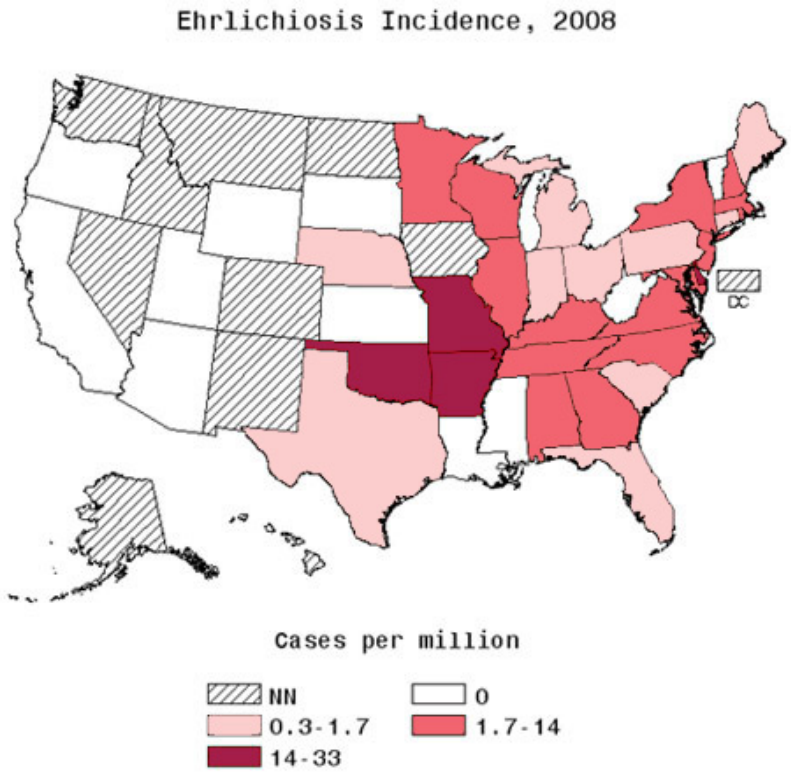


Figure 2. Annual reported incidence (per million population) for *E. chaffeensis* in the United States for 2008. (NN= Not notifiable) (CDC, 2012).

## TESTING OPTIONS

Laboratory testing presently includes hematologic, immunoserologic and molecular studies.

### Hematologic Testing

Blood smear analysis using EDTA-preserved blood samples has been a standard assay for detecting anaplasmosis and ehrlichiosis, and is a rapid assay available in most clinical laboratories. The assay primarily detects intracellular morulae of *A. phagocytophilum* (in granulocytes) and *E. chaffeensis* (in monocytes). The test detects morulae up to approximately two weeks from onset of symptoms and is highly specific, but lacks sensitivity compared with the molecular PCR assay.

### Immunoserologic Testing

Testing for IgG-class antibodies to *A. phagocytophilum* in human serum using the indirect fluorescent antibody (IFA) assay is a standard method for detecting pathogen exposure. Detectable antibodies appear shortly after symptoms first appear and remain elevated for greater than one

**How To Order This Test:**
**ANAPLASMA EHRLICHIA Nucleic Acid Test, NAT, BLOOD (84319)**
Keywords: Ehrlichia Molecular Detection, NAT, *Anaplasma phagocytophilum, Ehrlichia chaffeensis*

**Lab Test Code: ANENAT**
**Clinic (Clinical Order Manager):** Anaplasma Ehrlichia, NAT, (84319)
**HOSPITAL (Centricity):** Anaplasma Ehrlichia, NAT (84319)
Downtime: Write-In (Form I)

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

**Outreach** - Draw blood in a lavender-top (EDTA) tube(s), and send 5 mL of EDTA whole blood refrigerated. 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 Code:**
87798

**For questions please contact:**
Dr. Uphoff, Dr. Fritsche or Dr. Novicki at 800-222-5835.

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year. 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. Use of the PCR assay and/or smear analysis 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. Whether antibodies to *Ehrlichia* pathogens will be detected in the *Anaplasma* serology assay is unknown, but some degree of cross-reactivity may be expected due to their genetic relatedness.

**Molecular Testing**

During acute infection, molecular detection of bacterial DNA has been shown to be the most sensitive method of detection and superior to hematologic or immunoserologic methods. Molecular testing at Marshfield Labs is based on a method previously published by Bell and Patel (2005). This real-time PCR test targets a conserved region of the GroEL heat-shock protein operon amplifying a 359 base pair product from *A. phagocytophilum* and a 362 base pair product from the different *Ehrlichia spp*. Differentiation between the species is accomplished by melt temperature analysis of fluorescence resonance energy transfer (FRET) specific for *Anaplasma* and *Ehrlichia*. The sensitivity of this assay has been established to be 10 copies of the target DNA/mL of blood.

**Sample: Negative NAT Report**

Negative, no DNA matching that of *Ehrlichia chaffeensis, Ehrlichia ewingii, Ehrlichia muris-like*, or *Anaplasma phagocytophilum* was detected. These results do not exclude the presence of the organism or active/recent disease.

**Sample: Equivocal NAT Report**

This sample was tested in duplicate for *Anaplasma phagocytophilum* by PCR. The amount of *A. phagocytophilum* 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.

**Samples: Positive NAT Reports**

Positive for *Anaplasma phagocytophilum* by PCR. Positive results indicate presence of specific DNA from *Anaplasma phagocytophilum* and support the diagnosis of anaplasmosis.

Positive for *Ehrlichia ewingii/canis* by PCR. Positive results indicate presence of specific DNA from *Ehrlichia ewingii* or *Ehrlichia canis* and support the diagnosis of ehrlichiosis. *Ehrlichia ewingii* DNA is indistinguishable from that of *Ehrlichia canis* by this rapid PCR assay and a positive result indicates the presence of DNA from either of these two organisms.

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Both the negative and positive 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.

## REFERENCES:

Bell, C. A. and Patel, R. (2005). "A real-time combined polymerase chain reaction assay for the rapid detection and differentiation of *Anaplasma phagocytophilum, Ehrlichia chaffeensis,* and *Ehrlichia ewingii*." Diagnostic Microbiology and Infectious Disease 53(4): 301-306.

CDC (2006). "Diagnosis and Management of Tickborne Rickettsial Diseases: Rocky Mountain Spotted Fever, Ehrlichioses, and Anaplasmosis – United States." MMWR 55(Recommendation and Report 4): 1-27.

CDC (2012). "Annual Cases of Anaplasmosis in the United States." Retrieved 5/24/2012, from http://www.cdc.gov/anaplasmosis/stats/.

CDC (2012). "Annual Cases of Ehrlichiosis in the United States." Retrieved 5/25, 2012, from http://www.cdc.gov/Ehrlichiosis/stats/.

Pritt, B. S., Sloan, L. M., et al. (2011). "Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009." New England Journal of Medicine 365(5): 422-429.

# Revised Antibody Test for *Babesia microti* Infection

Thomas Fritsche, MD, Ph.D., FCAP, FIDSA ; Thomas Novicki, Ph.D., DABMM; Greg Simon, BS, MT(ASCP)

## SUMMARY

Beginning June 25, 2012, Marshfield Labs will offer an updated serological assay, **Babesia Microti IgG Antibody, IFA**, for the detection of the tick-borne pathogen *Babesia microti* from a single serum specimen.

**The Test Code is BABEAB**

This test will be offered in addition to traditional blood-smear analysis and PCR testing. It is most appropriate for testing early in the infection cycle during acute infection or to detect evidence of past exposure.

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## BACKGROUND

Babesiosis is an acute or chronic protozoan blood stream infection of humans and animals that is caused by *Babesia microti* (rarely other species), and is transmitted by the bite of the tick *Ixodes scapularis*, commonly known as the blacklegged deer or bear tick; less commonly, the infection may be acquired through blood transfusions. Infections may be subclinical to life-threatening with symptoms developing within weeks to months of a tick-bite exposure. Most serious infections occur in individuals who are immune-compromised, especially those who are asplenic or of advanced age.

The signs and symptoms of human babesiosis may mimic influenza and include fever, chills, body aches, weakness and fatigue along with physical findings of splenomegaly, hepatomegaly, and/or jaundice. Laboratory studies may reveal evidence of hemolytic anemia, thrombocytopenia, disseminated intravascular coagulation, proteinuria, hemoglobinuria, and elevated liver enzyme levels, BUN and creatinine. Severe cases may be associated with hemodynamic instability, acute respiratory distress, myocardial infarction, renal failure, hepatic compromise, altered mental status and death.

## TESTING OPTIONS

Laboratory testing presently includes hematologic, immunoserologic and molecular studies.

### Hematologic Testing

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 interpreting this parasite. The assay primarily detects intraerythrocytic 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, other laboratory tests that may prove helpful include antibody detection and PCR studies.

### Serologic Testing

The revised assay for IgG-class antibodies to *B. microti* in human serum uses the indirect fluorescent antibody (IFA) technique with *B. microti* as the antigen source, and may be useful in patients with a high likelihood of disease despite undetectable parasitemia. Antibodies usually appear shortly after symptoms are apparent and can be expected to remain elevated for some time. 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 and/or smear analysis 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. The assay is specific for *B. microti* infection, the most common species producing babesiosis in Wisconsin, and cannot be expected to display reactivity when a patient is infected with another *Babesia* species.

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**Molecular Testing**
During acute infection, molecular detection of *Babesia* DNA by PCR has been shown to be a more sensitive assay than either hematologic or immunoserologic methods.

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

## REFERENCES

CDC Babesiosis Provider Fact Sheet
http://www.cdc.gov/parasites/babesiosis/resources/babesiosis_hcp_fact_sheet.pdf

CDC Parasites – Babesiosis. Resources for Healthcare Professionals
http://www.cdc.gov/parasites/babesiosis/health_professionals/index.html

Herwaldt BL, Linden JV, Bosserman E, Young C, Olkowska D, Wilson M.
Transfusion-associated babesiosis in the United States: a description of cases.
Ann Intern Med. 2011. Oct 18;155(8):509-19.

Krause PJ, Telford SR III, Ryan R, et al. Diagnosis of babesiosis: Evaluation of a serologic test for the detection of *Babesia microti* antibody. J Infect Dis. 1994;169:923-926.