MAYO CLINIC LABORATORIES

Test Definition: TIKLB

Tick-Borne Panel, Molecular Detection, PCR, Blood

Overview

Useful For

Evaluating patients with suspected human monocytic ehrlichiosis, human granulocytic anaplasmosis, babesiosis, or *Borrelia miyamotoi* infection

Evaluating patients with a history of, or suspected, tick exposure who are presenting with fever, myalgia, headache, nausea, and other nonspecific symptoms

This test **should not be used** to screen healthy patients.

Profile Information

Test Id	Reporting Name	Available Separately	Always Performed
BABPB	Babesia species PCR, B	Yes	Yes
EPCRB	Ehrlichia/Anaplasma, PCR, B	Yes	Yes
BMIPB	Borrelia miyamotoi Detection, PCR, B	Yes	Yes

Testing Algorithm

For information see Acute Tick-Borne Disease Testing Algorithm.

Special Instructions

- Acute Tickborne Disease Testing Algorithm

Method Name

Real-Time Polymerase Chain Reaction (PCR)/DNA Probe Hybridization

NY State Available

Yes

Specimen

Specimen Type

Whole Blood EDTA

Specimen Required

Container/Tube: Lavender top (EDTA)

Specimen Volume: 1 mL

Collection Instructions:

1. Invert several times to mix blood
2. Send whole blood specimen in original tube. **Do not aliquot.**

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:

-Microbiology Test Request (T244)

-General Request (T239)

Specimen Minimum Volume

0.3 mL

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Whole Blood EDTA	Refrigerated	7 days	

Clinical & Interpretive

Clinical Information

In North America, ticks are the primary vectors of infectious diseases and rank second only to mosquitoes in disease transmission worldwide. In the United States, tick-borne diseases include Lyme disease, Rocky Mountain spotted fever, human monocytic ehrlichiosis, human granulocytic anaplasmosis, babesiosis, tularemia, relapsing fever, Colorado tick fever, and *Borrelia miyamotoi* infection.(1) Several of these diseases are transmitted by the same tick, and coinfections are occasionally seen. In particular, *Ixodes* species ticks are capable of transmitting the causative agents of Lyme disease (*Borrelia burgdorferi* and *Borrelia mayonii*), anaplasmosis (*Anaplasma phagocytophilum*), and babesiosis (*Babesia* species). These diseases are prevalent throughout the Northeastern and upper Midwestern states and parts of the Pacific Northwest.

Symptoms of the various tick-vectored diseases range from mild to life-threatening. Early symptoms, which include fever, aches, and malaise, do not aid in distinguishing the various diseases. Because early treatment can minimize or eliminate the risk of severe disease, early detection is essential, yet patients may not have developed distinctive symptoms to help in the differential diagnosis. A rapid tick-borne polymerase chain reaction panel can assist in identifying the pathogen, allowing treatment to be initiated.

While Lyme disease due to *B burgdorferi* is best detected through 2-tiered serologic testing, acute ehrlichiosis,

anaplasmosis, babesiosis, and *B miyamotoi* infection are best detected using molecular amplification assays. This tick-borne panel offers sensitive, specific, and rapid detection of the agents that cause these 4 diseases.

For information on the specific diseases, see the individual test information.

Reference Values

BABESIA SPECIES, MOLECULAR DETECTION, PCR
Negative

EHRLICHIA/*ANAPLASMA*, MOLECULAR DETECTION, PCR
Negative

BORRELIA MIYAMOTOI, MOLECULAR DETECTION, PCR
Negative

Reference values apply to all ages.

Interpretation

Borrelia miyamotoi:
A positive result indicates the presence of *Borrelia miyamotoi* DNA and is consistent with active or recent infection. While positive results are highly specific indicators of disease, they should be correlated with symptoms and clinical findings of tick-borne relapsing fever.

Ehrlichia/*Anaplasma*:
Positive results indicate presence of specific DNA from *Ehrlichia chaffeensis, Ehrlichia ewingii*, *Ehrlichia muris eauclairensis*, or *Anaplasma phagocytophilum* and support the diagnosis of ehrlichiosis or anaplasmosis.

Negative results indicate absence of detectable DNA from any of these 4 pathogens in specimens, but it does not exclude the presence of the organism or active or recent disease.

Since DNA of *E ewingii* is indistinguishable from that of *Ehrlichia canis* by this rapid polymerase chain reaction (PCR) assay, a positive result for *E ewingii*/*canis* indicates the presence of DNA from either of these 2 organisms.

Babesia:
A positive result indicates the presence of *Babesia* species DNA and is consistent with active or recent infection. While positive results are highly specific indicators of disease, they should be correlated with blood smear microscopy, serological results, and clinical findings.

A negative result indicates absence of detectable DNA from *Babesia* species in the specimen but does not always rule out ongoing babesiosis in a seropositive person since the parasitemia may be present at a very low level or may be sporadic.

Other tests to consider in evaluating a patient presenting with an acute febrile illness following tick exposure include serologic tests for Lyme disease (*Borrelia burgdorferi*) and molecular detection (PCR) for ehrlichiosis/anaplasmosis. For

patients past the acute stage of infection, serologic tests for these organisms should be ordered prior to PCR testing.

Cautions

This panel does not detect *Borrelia burgdorferi* or *Borrelia mayonii,* the causative agents of Lyme disease in the United States. While Lyme polymerase chain reaction testing (PBORB / Lyme Disease [*Borrelia burgdorferi*], Molecular Detection, PCR, Blood) can be useful for detecting acute infection with *B mayonii,* this organism has a limited geographic distribution (upper Midwestern United States) and is therefore not included in this panel. Serology is the preferred method for detection of *B burgdorferi.*

For more information, see the individual test IDs.

Supportive Data

Borrelia miyamotoi:
The following assay verification data supports the use of this assay for clinical testing.

Accuracy/Diagnostic Sensitivity and Specificity: Clinical Samples:
Sixty-two clinical EDTA blood specimens received in the clinical laboratory for *Ehrlichia/Anaplasma* polymerase chain reaction (PCR) were tested using the *Borrelia miyamotoi* PCR assay. Results were compared to the MDH 16S ribosomal RNA TaqMan assay. In addition, 2 retrospectively identified *B miyamotoi*-positive specimens were confirmed by the *B miyamotoi* PCR assay and the MDH TaqMan assay.

Spiking studies:
To supplement the clinical specimens, negative whole blood and cerebrospinal fluid (CSF) specimens were spiked with genomic or plasmid DNA of *B miyamotoi* near the limit of detection and tested in a blinded fashion. The sensitivity of the PCR assay was 100%, and the specificity with spiked specimens was 100%. The samples were extracted and tested in a blinded fashion.

Species Inclusivity:
-Four strains of *B miyamotoi* were detected by the PCR assay.
-Three other *Borrelia* species that cause human tick-borne relapsing fever (*Borrelia hermsii, Borrelia parkeri,* and *Borrelia turicatae*) were also detected at a melting temperature (Tm) below that of *B miyamotoi*. Thus, related *Borrelia* species may be detected and differentiated with this assay.
-Sixteen different strains from the *Borrelia burgdorferi* sensu lato group were not detected with this PCR assay.

Analytical Sensitivity/Limit of Detection:
-The limit of detection (LOD) is 2800 target copies/mL of whole blood or CSF.

Analytical Specificity:
-No PCR signal was obtained from the extracts of 31 bacterial, viral, parasitic, and fungal isolates from either similar organisms or organisms commonly found in the specimens tested.

Precision:
Interassay and intra-assay precision were 100%.

Reference Range:
The reference range of this assay is negative. This assay is designed to detect only species of clinical significance and is to be used for patients with a clinical history and symptoms consistent with tick-borne relapsing fever.

Reportable Range:
This is a qualitative assay, and the results are reported as negative or positive for *B miyamotoi* DNA.

Ehrlichia/Anaplasma:
The following validation data supports the use of this assay for clinical testing.

Accuracy/Diagnostic Sensitivity and Specificity:
Results from this real-time PCR assay on the LightCycler (LC PCR) were compared to those generated using conventional PCR assay for *Anaplasma phagocytophilum* on 127 unique, archived whole blood specimens (26 positive and 99 negative specimens by conventional PCR). Using the conventional PCR as the gold standard, the diagnostic sensitivity and specificity for detection of A phagocytophilum were 100%. In addition, 12 known *Ehrlichia chaffeensis* isolates and 2 *Ehrlichia ewingii* isolates (reference strains) were tested by the LC PCR and were positive.

Supplemental Data (Spiking Studies):
To supplement the above data, 30 negative whole blood samples were spiked with *A phagocytophilum* positive control plasmid at the LOD (10 copies/mcL). The 30 spiked specimens were run in a blinded manner along with 30 negative (non-spiked) specimens; 100% of the spiked specimens were positive, and 100% of the non-spiked specimens were negative.

Analytical Sensitivity/LOD:
The lower LOD of this assay for each of the species in EDTA blood is as follows:
-*A phagocytophilum*=approximately 10 targets per microliter
-*E chaffeensis*=approximately 5 targets per microliter
-*Ehrlichia muris eauclairensis*=approximately 100 targets per microliter
-*E ewingii/canis*=approximately 10 targets per microliter

Analytical Specificity:
No PCR signal was obtained from extracts of the following organisms: herpes simplex virus, Epstein-Barr virus, *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Bartonella henselae, Bartonella quintana, Rickettsia typhi, Rickettsia rickettsii, Toxoplasma gondii, Babesia microti* MN, *B microti* ATCC 53899, *B burgdorferi* ATCC 51990, *Ehrlichia risticii* ATCC VR-986, and *Anaplasma marginale*. Positive results were obtained from nucleic extracts of 2 *Ehrlichia canis* strains (patient strain and ATCC CRL-10390 strain), with a Tm of 49.5 degrees C (indistinguishable from *E ewingii*). A positive melting peak was also noted with *E muris* (ATCC VR-1411), but the Tm (55.24 degrees C) was easily distinguished from the Tm of the target organisms.

Precision:
Interassay precision was 97%, and intra-assay precision was 96%.

Reference Range:
Fifty whole blood specimens from normal donors were tested and found to be negative for targeted or detectable

Ehrlichia and *Anaplasma* species.

Reportable Range:
This is a qualitative assay, and results are reported as either negative or positive for targeted *Ehrlichia*/*Anaplasma* species (positive for *A phagocytophilum*, *E chaffeensis*, *E muris eauclairensis*, or *E ewingii*).

Babesia:
The following validation data supports the use of this assay for clinical testing.

Accuracy/Diagnostic Sensitivity and Specificity:
Ninety-six whole blood specimens were tested by this real-time PCR assay and another real-time PCR assay. Concordance was 99%.

Analytical Sensitivity/LOD:
The LOD established using whole organism spiked into specimen matrix (whole blood) is as follows:
-*Babesia microti*, ATCC PRA 99: 2670 target copies/mL
-*Babesia duncani* ATCC PRA 302: 1540 target copies/mL
-*Babesia* MO-1 positive patient DNA: 10,700 target copies/mL
-*Babesia divergens*-positive patient DNA: 5270 target copies/mL

Serial 10-fold dilutions of microscopy-positive specimens were also tested in a blinded fashion using conventional thick and thin blood films and the Mayo *Babesia* species PCR. The PCR was able to consistently detect two 10-fold dilutions lower than using microscopy.

Analytical Specificity:
No cross-reactivity was noted using a panel of 34 bacteria, viruses, parasites, and fungi were detected by the *Babesia* species PCR.

Precision:
Interassay and intra-assay precision were 100%.

Reference Range:
The reference range is negative. This was confirmed by testing 93 blood specimens from asymptomatic individuals for the presence of *Babesia* species by the *Babesia* species PCR assay. All 93 specimens were negative.

Reportable Range:
This test is a qualitative assay, and results are reported as positive or negative for *Babesia* species (*B microti*, *B duncani*, *B divergens*, and *B* MO-1).

Clinical Reference

Caulfield AJ, Pritt BS. Lyme disease coinfections in the United States. Clin Lab Med. 2015;35(4):827-846

Performance

Method Description

Borrelia miyamotoi:
The assay is performed on the Roche LightCycler (LC) 2.0 instrument, following DNA extraction on the Roche MagNA Pure. The LC 2.0 instrument amplifies and monitors the development of target nucleic acid (amplicon) after each cycle of polymerase chain reaction (PCR).

The DNA target for this PCR assay is a gene encoding the glycerophosphodiester phosphodiesterase (*glpQ*) gene specific to *Borrelia* species in the relapsing fever group. This gene is not found in *Borrelia* species that cause Lyme disease.

The specific base pair DNA target sequence is amplified by PCR. The detection of amplicon is based on fluorescence resonance energy transfer (FRET), which utilizes 1 hybridization probe with a donor fluorophore, fluorescein, at the 3' end and a second hybridization probe with an acceptor fluorophore, LC-Red 640, at the 5' end. When the target amplicon is present, the LC-Red 640 emits a measurable and quantifiable light signal at a specific wavelength. Presence of the specific organism nucleic acid is confirmed by performing a melting temperature analysis of the amplicon; the presence or absence of a melting peak in the appropriate temperature range is used to determine if a specimen is positive or negative.(Unpublished Mayo method)

Ehrlichia/*Anaplasma*:
Nucleic acid is extracted from the pathogens in blood using the automated MagNA Pure LC system. The extract is then transferred to individual self-contained capillary cuvettes for amplification. The LightCycler is an automated instrument that amplifies and monitors the development of target nucleic acid (amplicon) after each cycle of PCR. The DNA target for PCR assay is groEL, the open reading frame gene segment of the heat-shock protein operon (groEL), which is present at a frequency of 1 copy per organism in pathogenic species of *Anaplasma* and *Ehrlichia*. A specific base pair DNA target sequence is amplified by PCR. The detection of amplicon is based on FRET, which utilizes a hybridization probe with a donor fluorophore, fluorescein, at the 3' end and a second hybridization probe with an acceptor fluorophore, LC-Red 640, at the 5' end. When the target amplicon is present, the LC-Red 640 emits a measurable and quantifiable light signal at a specific wavelength. Presence of the specific organism nucleic acid may be confirmed by performing a melting curve analysis of the amplicon. Using features of the melting curve analysis, the assay primers and specific hybridization probes are able to detect and differentiate among *Anaplasma phagocytophilum*, *Ehrlichiosis chaffeensis*, *Ehrlichia muris eauclairensis*, and *Ehrlichia ewingii*/*canis*. Due to close proximity of the melting curves of *E ewingii* and *E canis*, this assay cannot distinguish between these 2 organisms.(Unpublished Mayo method)

Babesia:
Nucleic acid is extracted from EDTA whole blood using the automated MagNA Pure bead-based system (Roche Molecular Systems). The extract is then transferred to individual self-contained capillary cuvettes for amplification. The LightCycler is an automated instrument that amplifies and monitors the development of target nucleic acid (amplicon) after each cycle of PCR.

The DNA target for PCR assay is a gene encoding the nuclear small subunit ribosomal RNA.

This assay consists of 2 forward primers, 1 reverse primer, and 2 probes, which are specific for the *Babesia* species target DNA. The specific base pair DNA target sequence is first amplified by PCR using the target-specific primers. Amplicon is then detected during melting curve analysis using FRET probes, which utilizes 1 hybridization probe with a donor

fluorophore, fluorescein, at the 3' end and a second hybridization probe with an acceptor fluorophore, LC-Red 640, at the 5' end. Fluorescence is produced when the 2 probes anneal to the target sequence in close proximity to one another. The LC-Red 640 then emits a measurable and quantifiable light signal at a specific wavelength.(Burgess MJ, Rosenbaum ER, Pritt BS, et al. Possible transfusion-transmitted *Babesia divergens*-like/MO-1 in an Arkansas patient. Clin Infect Dis. 2017 Jun;64(11):1622-1625)

PDF Report
No

Day(s) Performed
Monday through Saturday

Report Available
Same day/1 to 4 days

Specimen Retention Time
7 days

Performing Laboratory Location
Rochester

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
87798 x4
87469
87468
87484
87478
87999 (if appropriate for government payers)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value

TIKLB	Tick-Borne DNA Panel, PCR, B	101647-6

Result ID	Test Result Name	Result LOINC® Value
618317	Babesia microti	88452-8
618318	Babesia duncani	88451-0
618319	Babesia divergens/MO-1	88450-2
618298	B. miyamotoi PCR, B	82475-5
618323	Anaplasma phagocytophilum	87558-3
618324	Ehrlichia chaffeensis	87559-1
618325	Ehrlichia ewingii/canis	87560-9
618326	Ehrlichia muris eauclairensis	87561-7