MAYO CLINIC LABORATORIES

Test Definition: EPCRB

Ehrlichia/Anaplasma, Molecular Detection, PCR, Blood

Overview

Useful For

Evaluating patients suspected of acute anaplasmosis or ehrlichiosis

This test **should not be** used for screening asymptomatic individuals.

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:

Preferred: Lavender top (EDTA)

Acceptable: Royal blue top (EDTA), pink top (EDTA), or sterile vial containing EDTA-derived aliquot

Specimen Volume: 1 mL

Collection Instructions: Send whole blood specimen in original tube (preferred).

Forms

If not ordering electronically, complete, print, and send Microbiology Test Request (T244) with the specimen.

Specimen Minimum Volume

0.3 mL

Reject Due To

Gross hemolysis	OK
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container

Whole Blood EDTA	Refrigerated	7 days	

Clinical & Interpretive

Clinical Information

Ehrlichiosis and anaplasmosis are emerging zoonotic tick-borne infections caused by *Ehrlichia* and *Anaplasma* species, respectively. These obligate intracellular, gram-negative rickettsial organisms infect leukocytes and cause a potentially serious febrile illness in humans.

Human granulocytic anaplasmosis (HGA), formerly known as human granulocytic ehrlichiosis, is caused by *Anaplasma phagocytophilum*, which is transmitted through the bite of an infected *Ixodes* species tick. The epidemiology of this infection in the US is similar to that of Lyme disease (caused by *Borrelia burgdorferi* and *Borrelia mayonii*) and babesiosis (caused primarily by *Babesia microti*), which all have the same tick vector. HGA is most prevalent in the upper Midwest and the Northeastern US.

Human monocytic ehrlichiosis (HME) is caused by *Ehrlichia chaffeensis,* which is transmitted by the Lone Star tick, *Amblyomma americanum*. Most cases of HME have been reported from the Southeastern and South-Central regions of the United States. *Ehrlichia ewingii*, the known cause of canine granulocytic ehrlichiosis, can occasionally cause an HME-like illness in humans. Clinical features and laboratory abnormalities are similar to those of *E chaffeensis* infection, and antibodies to *E ewingii* cross-react with current serologic assays for detection of antibodies to *E chaffeensis*.

Most recently, Mayo Clinic Laboratories detected a new species of *Ehrlichia* in patients with exposure to ticks in Wisconsin and Minnesota. This new pathogen, called *Ehrlichia muris eauclairensis*, causes a similar disease to ehrlichiosis due to *E chaffeensis* and *E ewingii* and may cause more severe disease in immunocompromised hosts.

Most cases of anaplasmosis and ehrlichiosis are subclinical or mild, but infection can be severe and life-threatening in some individuals. Fever, fatigue, malaise, headache, and other "flu-like" symptoms, including myalgias, arthralgias, and nausea, occur most commonly. Central nervous system involvement can result in seizures and coma.

Diagnosis may be challenging since the patient's clinical course is often mild and nonspecific. This symptom complex is easily confused with other illnesses such as influenza or other tick-borne zoonoses. Clues to the diagnosis of anaplasmosis/ehrlichiosis in an acutely febrile patient after tick exposure include laboratory findings of leukopenia, thrombocytopenia, and elevated serum aminotransferase levels. Intra-granulocytic morulae may be observed on peripheral blood smear in approximately 70% of cases of anaplasmosis, but intra-leukocytic morulare are rarely seen in human ehrlichiosis.

Definitive diagnosis is usually accomplished through polymerase chain reaction (PCR) and serologic methods, with the preferred method varying based on the time of presentation in relation to the onset of clinical symptoms. PCR is the most sensitive and specific method of detection in the first week of illness, whereas serology is the preferred method after this period.

The Mayo Clinic PCR assay is capable of detecting and differentiating *A phagocytophilum, E chaffeensis, E ewingii,* and *E muris eauclairensis.*

It is important to note that concurrent infection with *A phagocytophilum, Borrelia burgdorferi,* and *Babesia microti* is not uncommon, as these organisms share the same *Ixodes* tick vector. Additional testing for these pathogens, including Lyme disease serology, may be indicated.

Reference Values

Negative
Reference values apply to all ages.

Interpretation

Positive results indicate presence of specific DNA from *Ehrlichia chaffeensis, Ehrlichia ewingii*, *Ehrlichia muris eauclairensis* organism, 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 do not exclude the presence of these organisms or active or recent disease.

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

Cautions

This assay should only be used to test patients with signs and symptoms of ehrlichiosis or anaplasmosis.

A negative result does not indicate absence of disease.

Inadequate specimen collection or improper conditions for storage or transport may invalidate test results.

This test may detect DNA of *Ehrlichia canis* (reported to cause rare asymptomatic infection in Venezuela only).

This polymerase chain reaction test does not detect DNA of *Rickettsia* (formerly *Ehrlichia*) *sennetsu,* which has been reported to cause a rare mononucleosis-like illness in humans (in Japan and Malaysia).

Supportive Data

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

Accuracy/Diagnostic Sensitivity and Specificity:
Results from this real-time polymerase chain reaction (RT-PCR) assay on the LightCycle (LC) 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 *Anaplasma 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 *Anaplasma phagocytophilum* positive control plasmid at the limit of detection (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:
-*Anaplasma phagocytophilum*=approximately 10 targets per microliter
-*Ehrlichia chaffeensis*=approximately 5 targets per microliter
-*Ehrlichia muris eauclairensis* =approximately 100 targets per microliter
-*Ehrlichia 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 henselas, Bartonella quintana, Ricketssia typhi, Ricketssia rickettsii, Toxoplasma gondii, Babesia microti* MN, *Babesia microti* ATCC 53899, *Borellia 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 melting temperature (Tm) of 49.5 degrees C (indistinguishable from *Ehrlichia ewingii*). A positive melting peak was also noted with *Ehrlichia 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 *Anaplasma phagocytophilum*, *Ehrlichia chaffeensis*, *Ehrlichia muris eauclairensis,* or *Ehrlichia ewingii*).

Clinical Reference

1. Theel ES, Pritt BS. Ehrlichia, Anaplasma, and Related Intracellular Bacteria. In: Carroll KC, Pfaller MA, eds. Manual of Clinical Microbiology 13th ed. ASM Press; 2023
2. Pritt BS, Sloan LM, Johnson DK, et al. Emergence of a new pathogenic Ehrlichia species, Wisconsin and Minnesota, 2009. N Engl J Med. 2011;365(5):422-429
3. Johnson DKH, Schiffman EK, Davis JP, et al. Human infection with Ehrlichia muris-like pathogen, United States, 2007-2013. Emerg Infect Dis. 2015;21(10):1794-1799
4. Dixon EM, Branda JA, Clark SH, et al. Ehrlichiosis and Anaplasmosis subcommittee report to the Tick-Borne Disease working group. Ticks and Tick Borne Dis. 2021;12(6):101823

Performance

Method Description

Nucleic acid is extracted from the pathogens in blood using the automated MagNA Pure LC system. The extract is then transferred to a 96-well Lightcycler 480 dish for amplification. The LightCycler 480 is an automated instrument that amplifies and monitors the development of target nucleic acid (amplicon) after each cycle of polymerase chain reaction (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 fluorescence resonance energy transfer, 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 *Ehrlichia ewingii* and *Ehrlichia canis*, this assay cannot distinguish between these 2 organisms.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed

Monday through Sunday

Report Available

Same day/1 to 4 days

Specimen Retention Time

1 week

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

87468
87484
87798 x 2
87999 (if appropriate for government payers)

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
EPCRB	Ehrlichia/Anaplasma, PCR, B	87548-4

Result ID	Test Result Name	Result LOINC® Value
618323	Anaplasma phagocytophilum	87558-3
618324	Ehrlichia chaffeensis	87559-1
618325	Ehrlichia ewingii/canis	87560-9
618326	Ehrlichia muris eauclairensis	87561-7