Test Definition: LCMAL

Malaria, Molecular Detection, PCR, Varies

Overview

Useful For

Detection of *Plasmodium* DNA and identification of the infecting species

An adjunct to conventional microscopy of Giemsa-stained films, particularly in cases of low percent parasitemia or suboptimal parasite morphology

Detection and confirmatory identification of *Plasmodium* species: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium knowlesi*

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

Testing Algorithm

For more information see Malaria Laboratory Testing Algorithm.

Special Instructions

- Malaria Laboratory Testing Algorithm

Method Name

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

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

1. This test is **not** performed on a STAT basis and, therefore, should **not** be used as a primary screening test for malaria.
2. This test is used primarily for confirmation of a presumptive malaria diagnosis and determination of infecting *Plasmodium* species-particularly when the parasite morphology on traditional blood films is suboptimal.
3. Clients in the Rochester, MN area who are seeking a primary test for malaria and who can deliver the specimen within 4 hours of collection should order MAL / Rapid Malaria/*Babesia* Smear, Varies.
4. Laboratories that are unable to deliver a specimen within 4 hours of collection should perform an initial screen for malaria and other blood parasites in their laboratory prior to sending a specimen to Mayo Clinic Laboratories.
5. This test does **not** include blood smear examination/calculation of parasitemia. If calculation of percent parasitemia is also desired for cases that are positive for *Plasmodium* species, clients should order LMALP / Malaria PCR with Parasitemia Reflex, Varies.

Specimen Required

Both blood specimens and slides are required.

Specimen Type: Blood
Container/Tube: Lavender top (EDTA)
Specimen Volume: 4 mL
Collection Instructions:
1. Invert several times to mix blood.
2. Send specimen in original tube. **Do not aliquot.**

Specimen Type: Blood films
Container/Tube: Clean, grease-free slides in plastic slide container
Specimen Volume: 2 thin blood films and 2 thick blood films
Collection Instructions:
1. Ideally, blood films should be made directly from uncoagulated blood acquired via fingerstick. However, EDTA anticoagulated blood is also acceptable.
2. Prepare thin blood films as follows:
a. Prepare a thin film with a "feathered edge" that is no more than a single cell thick.
b. Allow the film to thoroughly air dry and then fix by briefly immersing in either absolute or 95% methyl alcohol.
c. Allow to air dry after fixation.
3. Prepare thick blood films as follows:
a. Place a large drop of blood (approximately the size of a dime and preferably from a fingerstick) on a slide.
b. Using a corner of a second slide, spread the drop in a circular motion while applying firm pressure to literally scratch the blood onto the carrier slide. This technique allows the blood to dry quickly and adhere well to the slide. Use approximately 20 circular sweeps with the second slide. The drop of blood should be about the size of a quarter when finished.
c. **Do not fix.** Air dry thoroughly (approximately 45 minutes) before placing in transport container.

Forms

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

Specimen Minimum Volume

Blood: 1 mL
Slides: See Specimen Required.

Reject Due To

Gross hemolysis	Reject
Gross lipemia	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Refrigerated (preferred)	7 days	

	Ambient	7 days	

Clinical & Interpretive

Clinical Information

Malaria is a mosquito-transmitted disease caused by apicomplexan parasites in the genus *Plasmodium.* It is an important cause of morbidity and mortality worldwide, with the World Health Organization (WHO) estimating 219 million cases and 435,000 malaria-related deaths in 2017. Malaria disproportionately affects individuals living in Africa (90% of cases), with individuals living in southeast Asia and the eastern Mediterranean regions next most affected. Malaria is also encountered outside of endemic regions, such as the United States, usually in returning travelers.

Malaria is caused primarily by 4 species of the protozoa *Plasmodium*: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale.* A fifth *Plasmodium* species, *Plasmodium knowlesi,* is a simian parasite that may be an important source of human infection in some regions of Southeast Asia. Differentiating *P falciparum* and *P knowlesi* from other species is important since both can cause life-threatening infections. In addition, *P falciparum* is typically resistant to many commonly used antimalarial agents, such as chloroquine.

Microscopy of Giemsa-stained thick and thin blood films is the standard laboratory method for diagnosis and differentiation of malaria parasites. Under optimal conditions, the sensitivity of the thick film microscopy is estimated to be 10 to 30 parasites per microliter of blood. However, microscopic diagnosis requires considerable expertise and may be insensitive or nonspecific when inadequate training and facilities are available. Furthermore, prolonged exposure to EDTA, transportation conditions, and prior use of antimalarial drugs may alter parasite morphology and negatively impact the ability to perform species identification by microscopy. Finally, *Babesia* parasites have a similar appearance to *P falciparum* ring forms (early trophozoites) on peripheral blood films, resulting in potential diagnostic confusion.

Polymerase chain reaction (PCR) analysis is an alternative method of malaria diagnosis that allows for sensitive and specific detection of *Plasmodium* species DNA from peripheral blood. PCR may be more sensitive than conventional microscopy in very low parasitemias and is more specific for species identification. It may be particularly useful when subjective microscopy does not permit certain identification of the species present. Malaria PCR can be used in conjunction with a traditional blood film or *Babesia* PCR when the clinical or morphologic differential includes both babesiosis and malaria. Examination of the thin film also allows for calculation of percent parasitemia, which can be used to predict prognosis and monitor response to treatment. This test does **not** include blood smear examination or calculation of parasitemia.

Reference Values

Negative

Interpretation

A positive result indicates the presence of *Plasmodium* nucleic acid and melting curve analysis indicates the infecting species.

Cautions

Malaria is potentially a life-threatening disease, and it is imperative to test for parasites as rapidly as possible. Therefore, this test is for confirmation only except for clients in the immediate Rochester, Minnesota area who can provide rapid delivery of specimens to Mayo Clinic Laboratories.

Assay may be negative in very low parasitemias.

Species of *Plasmodium* present in mixed infections may not be clearly delineated.

In some instances, the closely related species, *Plasmodium ovale* and *Plasmodium vivax*, cannot be differentiated from one another by this test. In this instance, results will be reported as "*P vivax/P ovale*." These 2 species have similar prognosis and treatment and can often be distinguished based on patient travel history.

This is a qualitative test only. If calculation of percent parasitemia is desired, order LMALP / Malaria PCR with Parasitemia Reflex, Varies.

This assay does not distinguish between residual nucleic acid (which may persist after adequate treatment) and viable intact parasites. It also does not distinguish between gametocytes (nonpathogenic forms that may be present in resolving infections) and virulent trophozoites.

Although the reference range is considered "negative" for individuals living in nonendemic areas, this assay may detect low-grade asymptomatic parasitemia from individuals exposed to malaria-endemic areas. However, this assay is designed to detect only *Plasmodium* species of clinical significance and is to be used for patients with a clinical history and symptoms consistent with malaria.

This polymerase chain reaction assay **does not** detect other parasites that may be present in the blood and have similar disease presentations including *Babesia* and *Trypanosoma* species.

Supportive Data

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

Accuracy/Diagnostic Sensitivity and Specificity:
A total of 160 clinical whole blood specimens were evaluated for the presence of *Plasmodium* species DNA or the 18S ribosomal RNA gene using this real-time polymerase chain reaction (PCR) assay. The assay detects and differentiates DNA of *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium knowlesi*. The results were compared to results of microscopy, a nested PCR method and sequencing. The specimens comprised 48 negative and 108 positive specimens (32 *P falciparum,* 8 *P malariae,* 20 *P ovale,* 45 *P vivax,* 2 unable to speciate by morphology, and 1 mixed infection of *P vivax/falciparum*). The sensitivity and specificity of the real-time *Plasmodium* assay compared to microscopy, nested PCR and sequencing was 99% and 94% respectively, with all species determinations by the PCR assay matching the original result. No *P knowlesi* clinical specimens were available, so spiking studies were performed. Thirty EDTA-anticoagulated blood specimens received in the laboratory for unrelated testing were spiked with *P knowlesi* plasmid near the limit of detection (50-100 targets per microliter) and tested in a blinded fashion with negative blood specimens. 100% concordance was achieved in the spiking studies.

Analytical Sensitivity/Limit of Detection:
The lower limit of detection of this assay is 10 to 50 DNA target copies per microliter in whole blood.

Analytical Specificity:
No PCR signal was obtained from extracts of 31 other bacterial, viral, rickettsial, and parasitic isolates that could be

found in whole blood and cause similar symptoms, including *Babesia, Borrelia, Anaplasma*, and *Ehrlichia* species.

Precision:
Interassay precision was 100% and the intra-assay precision was 100%.

Reportable Range:
This is a qualitative assay, and the results are reported as either negative or positive for the targeted *Plasmodium* species.

Clinical Reference

1. Mathison BA, Pritt BS: Update on malaria diagnostics and test utilization. J Clin Microbiol. 2017 Jul;55(7):2009-2017
2. Swan H, Sloan L, Muyombwe A, et al: Evaluation of a real-time polymerase chain reaction assay for the diagnosis of malaria in patients from Thailand. Am J Trop Med Hyg. 2005 Nov;73(5):850-854
3. World Health Organization (WHO). Malaria. WHO; Updated December 8, 2022. Accessed March 29, 2023. Available at www.who.int/news-room/fact-sheets/detail/malaria
4. Cox-Singh J, Davis T, Lee K, et al: *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life-threatening. Clin Infect Dis. 2008 January 15;46(2):165-171

Performance

Method Description

DNA from EDTA-anticoagulated whole blood is extracted and tested using real-time polymerase chain reaction on the LightCycler 2.0 instrument (Roche Applied Science) with primers and fluorescence resonance energy transfer (FRET) probes. A genus-specific primer set corresponding to 18S ribosomal RNA is used to amplify target sequence. One pair of FRET hybridization probes was designed for *Plasmodium falciparum* over a region containing base pair mismatches allowing for differentiation of other *Plasmodium* species by use of melting curve analysis, while a second probe set is specific for *Plasmodium knowlesi*.(Babady NE, Sloan LM, Rosenblatt JE, Pritt BS: Detection of *Plasmodium knowlesi* by real-time polymerase chain reaction. Am J Trop Med Hyg. 2009 Sept;81(3):516-518)

PDF Report

No

Day(s) Performed

Monday through Sunday

Report Available

Same day/1 to 3 days

Specimen Retention Time

7 days

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

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

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
LCMAL	Malaria PCR	47260-5

Result ID	Test Result Name	Result LOINC® Value
87860	Malaria PCR	47260-5