Malaria, Molecular Detection by Rapid Polymerase Chain Reaction
#87860

Clinical

Malaria is a major tropical disease infecting approximately 500 million people and causing 1.5 to 2.7 million deaths annually. Ninety percent of the deaths occur in sub-Saharan Africa and most of these occur in children <5 years old; it is the leading cause of mortality in this age group. This disease is also widespread in Central and South America, Hispaniola, the Indian subcontinent, the Middle East, Oceania, and Southeast Asia.

Malaria is caused by 4 species of the protozoa Plasmodium: Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale. Differentiating Plasmodium falciparum from other species is important since Plasmodium falciparum can cause life-threatening infections and is 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 speciation of malaria. Under optimal conditions, sensitivity of thick film microscopy is estimated to be 10 to 30 parasites per µl of blood. In resource-poor areas, microscopic diagnosis has been shown to be insensitive and nonspecific, especially when parasitemias are low or mixed infections are present. In field conditions, sensitivities and specificities as low as 71% to 72% have been reported.

A variety of PCR methods have been developed that increase sensitivity and specificity of detection as well as identification of mixed infections. In particular, use of PCR was shown to increase detection of placental malaria from 42% to 97% and asymptomatic parasitemia from 17% to 47%. 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.

Useful For

- Detection of Plasmodium and identification of the infecting species
- As an adjunct to conventional microscopy of Giemsa-stained smears
- For confirmatory identification of Plasmodium species

Interpretation

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

- Assay may be negative in very low parasitemias.
- Species of *Plasmodium* present in mixed infections may not be clearly delineated.
- This assay does not distinguish between residual nucleic acid (which may persist after adequate treatment) and viable intact parasites, nor between gametocytes (nonpathogenic forms that may be present in resolving infections) and virulent trophozoites.

References


Method


Specimen Required:

Both blood and slides are required for this test.

Blood

Draw blood in a lavender-top (EDTA) tube(s), and send 4.0 mL of EDTA whole blood in original VACUTAINERS(S). Invert several times to mix blood. Do not transfer blood to other containers. Forward unprocessed whole blood promptly at ambient temperature only. **Specimen cannot be frozen.**

Blood Films

Submit 2 thin blood films and 2 thick blood films made from fingerstick or drops of blood from needle following venipuncture. There should be no contact with any type of anticoagulant. To prepare blood films, use only clean, grease-free slides.

Thin Blood Films

Prepare with a “feathered edge”; should be no more than a single cell thick. Allow film to thoroughly air dry and then fix by briefly immersing it in either absolute or 95% methyl alcohol. Allow to air dry.

Send slides in plastic slide containers. Forward promptly at ambient temperature only.

Thick Blood Films

Place a large drop of blood (preferably from a fingerstick) on a slide. Using a corner of a second slide, spread drop by literally scratching carrier slide to make blood adhere. The drop of blood should start out the size of a dime and be about the size of a quarter when finished. Do not fix; air dry thoroughly (approximately 45 minutes) before placing in transport container.

Send slides in plastic slide containers. Forward promptly at ambient temperature only.

Note: If ordering electronically, no form is required with the specimen. If not ordering electronically, please complete and submit a “Microbiology Request Form” (Supply T244) with the specimen.

Reference Values:

Negative (no *Plasmodium* DNA detected)

Analytic Time:

Same day/1 day

Days Set Up:

Monday through Sunday; Varies

CPT Code:

87798
The pyruvate dehydrogenase complex (PDHC) catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA, which is an important entry point for 2-carbon acetyl units into the tricarboxylic acid (TCA) cycle (also called Kreb’s cycle or the citric acid cycle). The TCA cycle is a vital process that produces energy for cells.

PDHC is a multienzyme complex located in the inner mitochondrial membrane that consists of 6 different components: pyruvate decarboxylase (E1), dihydrolipoic transacetylase (E2), dihydrolipoyl dehydrogenase (E3), 2 regulatory enzymes (PDH kinase and PDH phosphatase), and E3-binding protein. PDHC is primarily regulated by a phosphorylation-dephosphorylation cycle catalyzed by PDH kinase or phosphatase, respectively.

In infants and children with PDHC deficiency, the most common features are delayed development and hypotonia. Seizures and ataxia are frequent features. Leigh disease, an inherited neurometabolic disorder, has been described in some of PDHC-deficient patients. Some infants with PDHC deficiency have congenital malformation in the brain including agenesis of the corpus callosum.

**Useful For**

- Evaluation of patients with a clinical suspicion of a pyruvate dehydrogenase complex deficiency or an energy metabolism disorder

**Interpretation**

When below-normal enzyme activities are detected, a detailed interpretation is given. This interpretation includes an overview of the results and their significance, a correlation to available clinical information, elements of differential diagnosis, and recommendations for additional biochemical testing.

**Cautions**

This assay is intended to detect decreases in total activity as a whole; it is not designed to pick up cases of PDH kinase or phosphatase deficiencies.
References


Method

PDHC activity is determined by measuring the (14)CO(2), the end product of the E1 component of PDHC from (1-[14]C) pyruvate. In skin fibroblasts, 90% to 95% of PDHC activity is inactivated. The enzyme is activated by pretreating fibroblast cells with dichloroacetate (DCA), an inhibitor of pyruvate dehydrogenase kinase. (Sheu KF, Hu CW, Utter MF: Pyruvate dehydrogenase complex activity in normal and deficient fibroblasts. J Clin Invest 1981 May;67[5]:1463-1471)

Specimen Required:
This test is not recommended for prenatal testing.

Submit only 1 of the following specimens:

Cultured Fibroblasts
1-T 75 or 2-T 25 flasks filled to neck with culture media. Maintain sterility and forward promptly at ambient temperature.

Skin Biopsy
Skin biopsy (4-mm punch). Collect sterile biopsy in Eagle’s medium essential medium with penicillin, streptomycin, and nystatin (Supply T115) supplied. (Specimen received in formalin or fixative preservative is not acceptable.) Send specimen refrigerated in a screw-capped, sterile container. Specimen cannot be frozen. Maintain sterility and forward promptly.

Note: Biopsy will be cultured at an additional charge. See #8482 “Fibroblast Culture”. Also see “Lysosomal Storage Disorders” in Special Instructions.

Pediatric collection instructions same as adult.

Reference Values:
PDHC, ACTIVATED
Range: 1.63-3.61 mU/mg protein
PDHC, INACTIVATED
Range: 0.18-2.18 mU/mg protein

Reference values apply to all ages.

Analytic Time: 49 days
Days Set Up: Varies
CPT Code: 82658