Falciparum/Pan Malaria Test
Rapid Diagnostic Test for Detecting Malaria Infection

Name and Intended Use

Falciparum/Pan Malaria Test is a visual, qualitative immunoassay for *in vitro* detection of plasmodium Lactate Dehydrogenase (pLDH), a protein specific to parasites of the genus Plasmodium. The test is intended as an aid to diagnosis of malaria infection in humans.

Summary and Explanation of the Test

Malaria is a serious, sometimes fatal, parasitic disease characterized by fever, chills and anemia and is caused by a parasite transmitted from one human to another by the bite of infected Anopheles mosquitoes. There are four species of parasite that infect humans: *Plasmodium falciparum, P. vivax, P. ovale, and P. malariae.* The disease occurs in more than 90 countries worldwide, and it is estimated that there are over 500 million clinical cases and 2.7 million malaria caused deaths per year. At present, malaria is diagnosed by microscopic examination of blood. **Falciparum/Pan Malaria Test** is a rapid, simple immunoassay that does not require a high degree of technical expertise and can be performed under resource-limited conditions.

Biological Principles of the Procedure

Falciparum/Pan Malaria Test detects pLDH, an enzyme produced by the four species of Plasmodium known to cause malaria in humans. A small sample of blood is added to the sample window and lysis buffer is added to a second window. The buffer contains a detergent that lyses the red blood cells and releases the cell contents. If pLDH is present, this forms a complex with the colloidal gold anti-pLDH conjugate that is dried onto the test strip. The liquid migrates through the nitrocellulose, and if colloidal gold – antibody – pLDH complex is present, this binds to a second anti-pLDH antibody immobilized on the nitrocellulose, forming a visible purple line. To insure assay validity, a second control line is incorporated into the assay device.

Materials Provided

Falciparum/Pan Malaria Test kit contains the following components to perform the assay:

1. Test Device (cassette)
2. Sample loop (5 microliter)
3. Lancet
4. Lysis buffer
5. Instruction manual
6. Disinfectant pad

Accessories (required but not provided):

1. Micropipette (if loop not used)
2. Timer

Storage and Stability

The test devices are sealed in a moisture-proof foil laminate pouch, containing desiccant. These should be stored in the coolest and driest area available, preferably at 2-35°C. The kit has a shelf life of 12 months from the date of manufacture. The devices should not be frozen and must be protected from exposure to humidity; once a pouch is opened, the device should be used within one hour.

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Precautions

To obtain reproducible results, the following instructions must be followed:

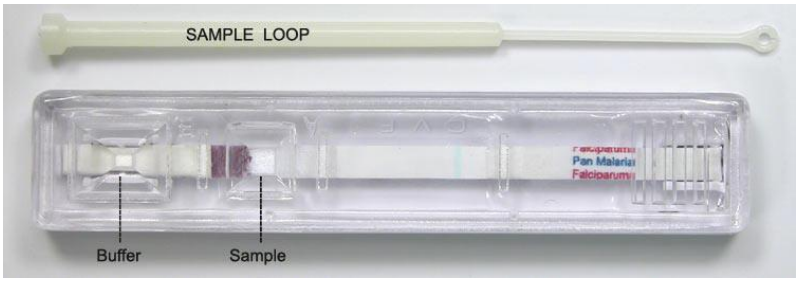
1. For *in vitro* diagnostic use only with whole blood. Not for use with serum or plasma samples.
2. Use disposable gloves while handling potentially infectious material and performing the assay.
3. Do not use the kit beyond the expiration date.
4. Do not eat, smoke, or drink while handling specimens.
5. Do not combine reagents from different batch numbers as the components are optimized for individual batch to give best results.
6. Clean up spills thoroughly using an appropriate disinfectant.
7. Do not open the foil pouch until it attains room temperature to prevent formation of condensation.
8. For best results, follow the test procedure and storage instructions strictly.

Specimen Collection and Storage

1. Collect whole blood in a clean container containing anti-coagulant (EDTA, citrate or heparin) by venipuncture. Fresh samples are preferred for testing. If samples are not immediately tested, they should be stored at 4-8°C for not more than 3 days. Samples may be frozen at –20°C, but should not be repeatedly frozen and thawed.
2. Fresh blood from a finger prick may also be used as a test sample.
3. Clotted samples or samples with microbial contamination must not be used.

Test Procedure

1. Bring the complete kit and sample to be tested to room temperature prior to testing. Once the device pouch is opened, it must be used within one hour.
2. Using the sample loop provided, or a suitable micropipette, place one loop of blood (5 microliters) into the sample window of the device. If using the loop, press gently against the bottom of the sample window and allow theblood to flow out of the loop.
3. Add 150 µL (5 drops) of Lysis Buffer to the buffer window as shown.
4. As the blood lyses, it will flow onto the nitrocellulose, coloring it red. As additional buffer flows through the nitrocellulose, the red color will clear, and test and/or control lines will appear. This typically requires 15-20 minutes. The test should be read as soon as the red color of blood has cleared from the nitrocellulose.



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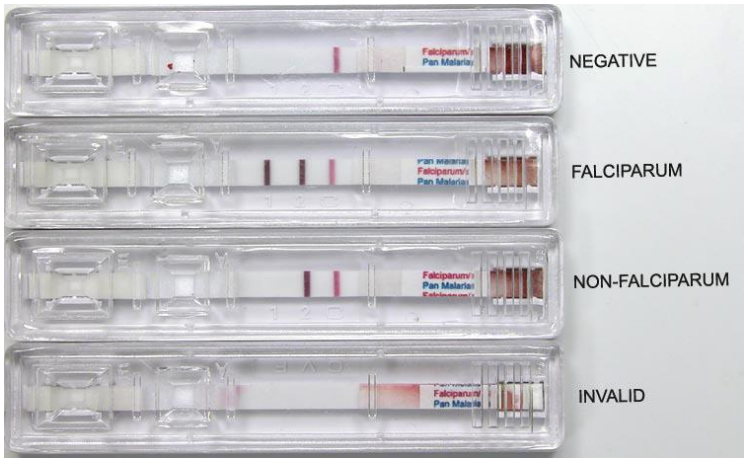
Interpretation of Results

As shown below, a reactive test shows as one or two colored lines in the test area, and one in the control area. Two lines in the test area indicate falciparum malaria. One line in the test area indicates non-falciparum malaria. A non-reactive test shows only one colored line in the control area. If no lines are visible, the test is invalid.



Performance Characteristics

The test can detect parasitemia levels of 100 parasites per microliter of blood (0.002% parasitemia) based on evaluation of microscopically characterized samples of *P. falciparum* infected blood. The test has been evaluated with positive and negative clinical samples with results as follows:

Sample	Total Number	Positive	Negative	Sensitivity (%)	Specificity (%)
Malaria negative	450	0	450	-	100
P. falciparum Positive	105	96	9	91.4 **	-
P. vivax Positive	66	65	1	98.5	-
P. ovale Positive	15	15	0	100	-
P.malariae Positive	10	10	0	100	-

** Missed specimens had parasitemia less than 100 parasites per microliter. Sensitivity was 99% at 100 parasites/µL or higher.

Limitations and Interferences

1. The test procedure, precautions and interpretation of results for the test must be followed strictly.
2. As with all diagnostic tests, the test result must always be consistent with clinical findings.
3. The results are to be interpreted within the epidemiological, clinical, and therapeutic context. When it is indicated, the parasitological techniques of reference should be considered for confirmation (microscopic examination of thin and thick blood smears).

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