

Science with a Mission, Inc. (SMI)
602 Massapoag Avenue
Sharon, MA 02067
Tel. or Fax (781) 784-6907
Web Address: www.sciencewithamission.org

**Malaria Pf (HRP II) / pan (pLDH)
Antigen Detection Test Cassette
(Whole Blood)**

*A rapid test for the qualitative detection of Human
Malaria antigen in whole blood.*

For professional in vitro test use only.

Intended Use

For the rapid qualitative determination of Malaria plasmodium falciparum specific histidine rich protein-II (Pf HRP-II) and Malaria plasmodium lactate dehydrogenase (pLDH) in human blood as an aid in the diagnosis of Malaria infection.

Summary

Malaria is a serious parasitic disease characterized by fever, chills, and anemia and is caused by parasites that are transmitted from one human to another by the bite of infected Anopheles mosquitoes. There are four types of parasites that cause malaria in humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. In humans, the parasites (called sporozoites) migrate to the liver where they mature and release another form, the merozoites. The disease now occurs in more than 90 countries worldwide, and it is estimated that there are over 500 million clinical cases and maybe 2 million malaria-caused deaths per year. At the present, malaria is diagnosed by looking for the parasites in a drop of blood. The blood is put onto a microscope slide and stained so that the parasites will be visible under a microscope.

Our one-step Malaria Pf (HRP II) / pan (pLDH) antigen detection test contains a membrane strip, which is pre-coated with two monoclonal antibodies as two separate lines across a test strip. One monoclonal antibody (test line 1) is specific to the *P. falciparum* histidine rich protein-II (Pf HRP-II) and another monoclonal antibody (test line 2) is pan

specific to the lactate dehydrogenase of all four Plasmodium species (*falciparum*, *vivax*, *malariae*, *ovale*). The conjugate pad contains monoclonal antibodies conjugated to colloidal gold, which are specific to Pf HRP-II and specific to the lactate dehydrogenase of *Plasmodium species*.

So this Malaria test is designed for the differential diagnosis between *Plasmodium falciparum* and all other *Plasmodium species*

There is also a control line, giving you confidence in the results you obtain.

Precautions

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- Humidity and temperature can adversely affect results.

Storage & Stability

The kit can be stored at room temperature or refrigerated (2-30 °C). The test device is stable through the expiration date printed on the sealed pouch. The test device must remain in the sealed pouch until use. **DO NOT FREEZE.** Do not use beyond the expiration date.

Materials

Materials provided:

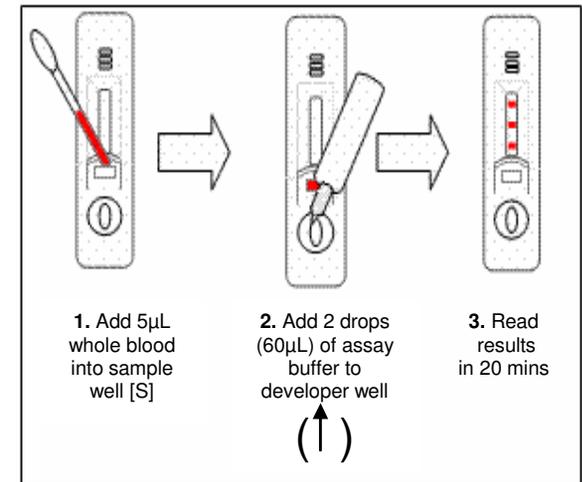
- Test Cassette
- Instructions
- 5uL transfer pipette
- Buffer

Materials not provided:

- Timer

Specimen Collection & Preparation

- 1) Clean the area of the fingertip to be lanced with an alcohol swab and allow to dry.
- 2) Squeeze the end of the fingertip and pierce with a sterile lancet.
- 3) Wipe away the first drop of blood with sterile gauze or cotton.
- 4) Hold the transfer pipette (provided) horizontally and touch the tip to the drop of blood. The blood will fill the pipette to the line and stop automatically. The sample is ready to be placed on the cassette.



Directions for Use:

- 1) Add the 5 µl of whole blood just collected with the transfer pipette, into sample well [S] of the cassette. This well is purple in color. You may need to touch the tip of the transfer pipette to the well bottom in order to get all the blood out of the pipette.
- 2) Add two drops (approximately 60 µL) of assay buffer into the well marked with an arrow [↑]
- 3) Read the test result in 20 min.

Interpretation of Results:

1) *P. falciparum* positive reaction

The presence of three colored lines (c, 1, and 2) indicates a positive result for *P. falciparum*. The control line tells you the test worked properly and you can trust the result. The line at 2 indicates that the patient has some form of malaria as indicated by the presence of pLDH in the blood sample. The line at 1 indicates that Pf HRP-II was present in the sample. This is only the case when the patient has *P. falciparum* malaria.

NOTE: It is possible for you to have lines at c and 1 ONLY (no line at 2) for some Pf-positive patients. This may be due to patients self-medicating which "inactivates" the pLDH making it undetectable. In any case, patients with a line at c and 1 should be treated for *P. falciparum* malaria.

2) *P. vivax* or *P. malariae* or *P. ovale* positive reaction

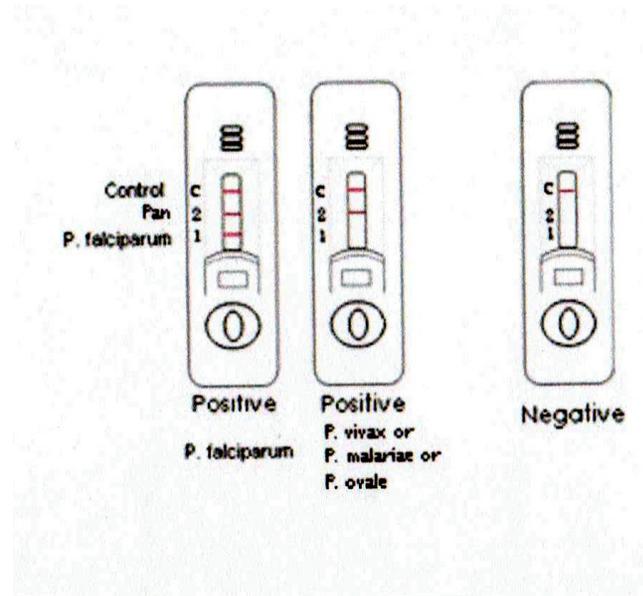
The presence of two colored lines (c and 2) indicates a positive result for *P. vivax* or *P. malariae*, or *P. ovale*. The control line tells you the test worked properly and you can trust the result. The line at 2 indicates that the patient has some form of malaria as indicated by the presence of pLDH in the blood sample. The absence of a line at 1 indicates that they do not have *P. falciparum*.

3) Negative reaction

The presence of only one line at the control line (c) indicates a negative result. The test worked properly and you can trust the results, but the patient does not have any form of malaria.

4) Invalid

The test is invalid if the control line does not appear. If this occurs, the test should be repeated using a new cassette.



Limitations

- 1) The test procedure, precautions and interpretation of results for this test must be followed when testing.
- 2) Anti-coagulants such as heparin, EDTA, and citrate do not affect the test result.
- 3) This test kit detects Plasmodium HRP-II and lactate dehydrogenase in patient's whole blood and is useful as a screening procedure of malaria diagnosis.
- 4) The test is limited to the detection of antigen to Malaria *Plasmodium* sp. Although the test is very accurate in detecting HRP-II and pLDH, a low incidence of false results can occur. Other clinically available tests are required if questionable results are obtained. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
- 5) Please note we had one group (in Tanzania) find that line 2 sometimes took closer to 25 minutes to form. This was only the case when the patient was positive for *P. falciparum*. After 20 minutes there was a clear line at C and 1, but the line at 2 took longer to form.

Bibliography

- 1) Leonard K. Basco, Frederique Marquet, Michael M. Makler, and Jacques Le Bras. : *Plasmodium falciparum* and *Plasmodium vivax*: Lactate Dehydrogenase Activity and its Application for in vitro Drug Susceptibility Assay. *Experimental Parasitology* 80, 260-271 (1995)
- 2) David L. Vander Jagt, Lucy A. Hunsaker and John E. Heidrich: Partial Purification and Characterization of Lactate Dehydrogenase from *Plasmodium falciparum*. *Molecular and Biochemical Parasitology*, 4 (1981) 255-264.
- 3) David J. Bzik, Barbara A. Fox and Kenneth Gonyer : Expression of *Plasmodium falciparum* lactate dehydrogenase in *Escherichia coli* *Molecular and Biochemical Parasitology*, 59(1993) 155-166
- 4) Cameron R. Dunn, Mark J. Banfield, John J. Barker, Christopher W. Highm, Kathleen M. Moreton, Dilek Turgut-Balik, R. Leo Brady and J. John Holbrook. The Structure of lactate dehydrogenase from *Plasmodium falciparum* reveals a new target for anti-malarial design. *Nature Structural Biology* 3(11)1996, 912-915
- 5) Howard, RJ, et al. The secretion of a Malaria Histidine-rich Protein (Pf HRP II) from *Plasmodium falciparum*-infected Erythrocytes. *J. Cell Biol.*, (1986)103, 1269-1277
- 6) Rock, EP, et al. Comparative Analysis of the *Plasmodium falciparum* Histidine-Rich Proteins HRP-I, HRP-II, and HRP-III in Malaria Parasites of Diverse Origin. *Parasitol.*, (1987) 95:209-227
- 7) Parra, ME, et al, Identification of *Plasmodium falciparum* Histidine-Rich Protein 2 in the plasma of Humans with Malaria. *J. Clin. Microbiol.*, (1991) 29:1629-1634
- 8) Rodriguez-del Valle, M., et al, Detection of Antigens and Antibodies in the Urine of Humans with *Plasmodium falciparum* Malaria. *J. Clin. Microbiol.*, (1991) 29:1236-1242