Malaria Pf/Pv Rapid Test (Cassette)

(Whole Blood)

The Malaria Pf/Pv Ag Rapid Test is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of *Plasmodium falciparum* (*Pf*) and *vivax (Pv)* antigen in human blood specimen. This device is intended to be used as a screening test and as an aid in the diagnosis of infection with plasmodium. Any reactive specimen with the Malaria Pf/Pv Ag Rapid Test must be confirmed with alternative testing method(s) and clinical findings.

INTRODUCTION

Malaria is a mosquito-borne, hemolytic, febrile illness that infects over 200 million people and kills more than 1 million people per year. It is caused by four species of *Plasmodium*: *P. falciparum, P.vivax, P. ovale, and P. malariae*. These plasmodia all infect and destroy human erythrocytes, producing chills, fever, anemia, and splenomegaly. *P. falciparum* causes more sever disease than

the other plasmodial species and accounts for most malaria deaths. *P. falciparum and P. vivax* are the most common pathogens, however, there is considerable geographic variation in species distribution1.

Traditionally, malaria is diagnosed by the demonstration of the organisms on Giemsa stained thick smears of peripheral blood, and the different species of plasmodium are distinguished by their appearance in infected erythrocytes1. The technique is capable of accurate and reliable diagnosis, but only when performed by skilled microscopists using defined protocols2, which presents major obstacles for the remote and poor areas of the world.

The Malaria Pf/Pv Ag Rapid Test is developed for solving these obstacles. It utilizes antibodies specific to *P. falciparum* Histidine Rich Protein-II (pHRP-II) and to *P. vivax* Lactate Dehydrogenase (Pv-LDH) to simultaneously detect and differentiate infection with *P. falciparum* and *P. vivax*-5. The test can be performed by untrained or minimally skilled personnel, without laboratory equipment

PRINCIPLE

During the assay, an adequate volume of the blood specimen is dispensed into the sample well (S) of the test cassette, a lysis buffer is added to the buffer well (B). The buffer contains a detergent that lyses the red blood cells and releases various antigens, which migrate by capillary action across the strip held in the cassette. Pv-LDH if presents in the specimen will bind to the Pv-LDH-gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-Pv-LDH antibody, forming a burgundy colored T1 band, indicating a *Pv* positive test result.

Alternatively, pHRP-II if presents in the specimen will bind to the pHRP-II-gold conjugates. The immunocomplex is then captured on the membrane by the pre-coated anti-pHRP-II antibodies, forming a burgundy colored T2 band, indicating a *Pf* positive test result.

Absence of any T bands (T1 and T2) suggests a negative result. The test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex of goat antimouse IgG / mouse IgG (anti-Pv-LDH and anti-pHRP-II)-gold conjugates regardless of the color development on any of the T bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

MATERIALS SUPPLIED

1.Test device 2 Pipette dropper 3.Desiccant 4.Buffer 5.Package Insert

MATERIAL REQUIRED BUT NOT PROVIDED

1. Clock or Timer2. Lancing device for whole blood test

STORAGE AND STABILITY

All reagents are ready to use as supplied. Store unused test device unopened, preferably at 2°C-30°C.

Do not expose the kit over 40°C. Do not freeze the kit. Ensure that the test device is brought to room temperature before opening. The test device is stable through the expiration date printed on the sealed pouch if it is stored at 2°C-30°C.

WARNINGS AND PRECAUTIONS

1. For professional In Vitro diagnostic use only.
2. Warning: the reagents in this kit contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
3. Do not use it if the tube/pouch is damaged or broken.
4. Test is for single use only. Do not re-use under any circumstances.

SPECIMEN COLLECTION

Consider any materials of human origin as infectious and handle them with standard biosafety procedures.

Collect whole blood in a clean container containing anti-coagulant (EDTA, citrate or heparin) by venipuncture. Blood can be obtained by finger tip puncture as well. Whole blood specimen should be stored in refrigeration (2°C-8°C) if not tested immediately for up to 3 days. The specimen should be frozen at -20°C for longer storage. Avoid repeat freeze and thaw.

TEST PROCEDURE

1. Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed. Blood will be hemolyzed after thawing.

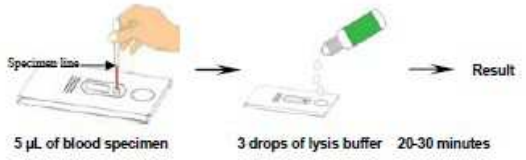
2. When ready to test, open the pouch at the notch and remove device. Place the test device on a clean, flat surface.

3. Be sure to label the device with specimen's ID number.

4. Fill in the mini plastic dropper with the blood specimen not to exceed the specimen line as showed in the following image. The volume of the specimen is around 5 µL.

Note: Practice a few times prior to testing if you are not familiar with the mini dropper. For better precision, transfer specimen by pipette capable to deliver 5µL of volume. Holding the dropper vertically, dispense all of the specimen into the center of the sample well making sure that there are no air bubbles.

Then add 3 drops (about 100-150 µL) of Lysis Buffer immediately.



5. Set up timer.

If preferred, after 5 minutes of adding specimen and buffer, you may add one more drop of Lysis Buffer to help the background become clearer.

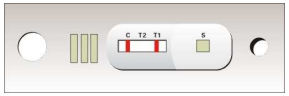
6. Results can be read in 20 to 30 minutes. It may take more than 20 minutes to have the background become clearer.

Don't read results after 30 minutes. To avoid confusion, discard the test device after interpreting the result

INTERPRETATION OF RESULTS

1. POSITIVE:

1.1 In addition to the presence of C band, if only T1 band is developed, the test indicates for the presence of Pv-LDH antigen.

The result is Pv positive. 

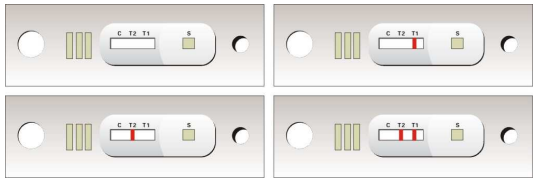
1.2 In addition to the presence of C band, if only T2 band is developed, the test indicates for the presence of pHRP- II antigen. The result is Pf positive.

1.3 In addition to the presence of C band, both T1 and T2 bands are developed, the test indicates for the presence of both Pv-LDH and pHRP-II antigens. The result is both Pv and Pf positive

Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

2. NEGATIVE: If only the C band is present, the absence of any burgundy color in the both T bands (T1 and T2) indicates that no anti-plasmodium antigens are detected. The result negative.

3. **INVALID:** If no C band is developed, the assay is invalid regardless of any burgundy color in the T bands as indicated below. Repeat the assay with a new device.



QUALITY CONTROL

Using individual Malaria (Pf/v) One Step Rapid Tests as described in the Assay Procedure above, run 1 positive control and 1 Negative Control to monitor test performance:

1. A new operator uses the kit, prior to performing testing of specimens.
2. A new test kit is used.
3. A new shipment of kits is used.
4. The temperature used during storage of the kit fall outside of 2°C-30°C.
5. The temperature of the test area falls outside of 15°C-30°C.

LIMITATIONS

1. The Assay Procedure and the Test Result Interpretation must be followed closely when testing the presence of plasmodium protozoa antigen in whole blood from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Malaria (Pf/Pv) One Step Rapid Test is limited to the qualitative detection of plasmodium protozoa antigen in whole blood. The intensity of the test band does not have linear correlation with the antigen titer in the specimen.
3. In the case of co-infection with Pf and any of the other three plasmodia, both T1 and T2 band will be developed. Thus, interpret the result cautiously when both T1 and T2 bands are visible.
4. A negative result for an individual subject indicates absence of detectable plasmodium protozoa antigen. However, a negative test result does not preclude the possibility of exposure to or infection with plasmodium protozoa.
5. A negative result can occur if the quantity of the plasmodium protozoa antigen present in the specimen is below the detection limits of the assay, or the antigen that are detected are not present during the stage of disease in which a sample is collected.
6. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
7. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

PERFORMANCE CHARACTERISTICS

1. Clinical Performance with Pf positive specimen

A total of 211 samples from susceptible subjects were tested by the Malaria (Pf/Pv) One Step Rapid Test and by thick blood smear test.

Method		Smear Test		Total Results
Malaria Test Device	Results	Positive	Negative	
	Positive	21	3	24
	Negative	1	189	190
Total Results		22	192	214

Relative Sensitivity: 95.5%, Relative Specificity: 98.4%, Overall Agreement: 98.1%

2. Clinical Performance with Pv positive specimen

A total of 224 samples from susceptible subjects were tested by The Malaria (Pf/Pv) One Step Rapid Test and by thick blood smear test.

Method		Smear Test		Total Results
Malaria Test Device	Results	Positive	Negative	
	Positive	12	3	15
	Negative	0	209	209
Total Results		12	212	224

Relative Sensitivity: 100%, Relative Specificity: 98.5%, Overall Agreement: 98.7%

REFERRENCE

1. Malaria, p. 421-424. Chapter 9. Infectious and Parasitic Diseases. Rubin E., Farber JL: Pathology, 2nd ed. 1994. J.B. Lippincott, Philadelphia.
2. Cooke AH, Chiodini PL, Doherty T, et al, Am J Trop Med. Hyp, 1999, Feb: 60(2):173-2
3. Guthmann JP, et al: Trans R Soc Trop Med Hyg. 2002, 96(3):254-7
4. Kar I, Eapen A, Adak T, Sharma VP, Indian J Malariol. 1998, 35(3):160- 2