

## RAPID TEST FOR *P. FALCIPARUM* MALARIA

(Device)

Cat# MAL025, MAL010

For Laboratory use only



### INTRODUCTION

Rapid Test for *P. falciparum* Malaria is a rapid, qualitative, two site sandwich immunoassay for the determination of *P. falciparum* specific histidine rich protein - 2 (Pf. HRP-2) in whole blood samples.

### SUMMARY

Four species of the Plasmodium parasites are responsible for malaria infections in humans viz. *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Of these *P. falciparum* is the most prevalent and severe species that is responsible for most of the morbidity and mortality worldwide. Early detection of *P. falciparum* malaria is of paramount importance due to incidence of cerebral malaria and drug resistance associated with it. Pf. HRP-2 is a water soluble protein that is released from parasitised erythrocytes of infected individuals and is specific to the *P. falciparum* species.

Rapid Test for *P. falciparum* Malaria detects the presence of Pf. HRP-2 in whole blood specimen and is a sensitive and specific test for the detection of *P. falciparum* malaria.

### PRINCIPLE

Rapid Test for *P. falciparum* Malaria is a rapid test for the detection of *P. falciparum* malaria that utilizes the principle of immunochromatography. As the test sample flows through the membrane assembly of the device after addition of the clearing buffer, the colored monoclonal anti Pf. HRP-2 (IgG) colloidal gold conjugate antisera complexes the Pf. HRP-2 in the lysed sample. This complex moves further on the membrane to the test region where it is immobilized by the monoclonal anti Pf. HRP-2 (IgM) antisera coated on the membrane leading to formation of a pink-purple colored band which confirms a positive test result. Absence of this colored band in the test region indicates a negative test result. The unreacted conjugate and unbound complex (if any) move further on the membrane and are subsequently immobilized by anti rabbit antibodies coated on the membrane at the control region, forming a pink-purple band. This control band serves to validate the test performance.

### REAGENTS AND MATERIALS SUPPLIED

Each kit contains:

A. Individual pouches, each containing:

1. Device: Membrane assembly predispensed with anti Pf. HRP-2 (IgG) antisera-colloidal gold conjugate, rabbit IgG-colloidal gold conjugate and anti Pf. HRP-2 (IgM) antisera and anti rabbit antisera at the respective regions.
2. Desiccant pouch.
3. Pipette: 5µl sample applicator.

B. Clearing buffer in a dropper bottle.

C. Package insert.

### OPTIONAL MATERIAL REQUIRED

Calibrated micro pipette capable of delivering 5 µl sample accurately.

### STORAGE AND STABILITY

The test kit may be stored between 4-45°C till the duration of the shelf life as indicated on the pouch / carton. DO NOT FREEZE.

### NOTE

Read the instructions carefully before performing the test.

For Laboratory use only. For in vitro diagnostic use only. NOT FOR MEDICINAL USE.

Do not use beyond expiry date.

Do not intermix reagents from different lots.

Handle all specimens as potentially infectious.

Follow standard biosafety guidelines for handling and disposal of potentially infective material.

### SPECIMEN COLLECTION AND PREPARATION

Fresh anti coagulated whole blood should be used as test sample and EDTA or Heparin or Oxalate can be used as suitable anticoagulant. The specimen should be collected in a clean glass or plastic container. If immediate testing is not possible then specimen may be stored at 2 - 8°C for upto 72 hours before testing. Clotted or contaminated blood samples should not be used for performing the test. Fresh blood from finger prick / puncture may also be used as a test specimen.

### TEST PROCEDURE AND INTERPRETATION OF RESULTS

1. Bring the Rapid Test for *P. falciparum* Malaria kit components to room temperature before testing.
2. In case the pouch has been stored at 2 - 8°C allow at least 30 minutes for the device to come to room temperature.
3. Open the pouch and retrieve the device, sample applicator and desiccant. Check the colour of the desiccant. It should be blue. If it has turned colourless or pink discard the device and use another device. **Once opened, the device must be used immediately.**
4. Tighten the vial cap of the clearing buffer provided with the kit in the clockwise direction to pierce the dropper bottle nozzle.
5. Evenly mix the anti coagulated blood sample by gentle swirling. Dip the sample applicator into the sample. Ensuring that a loop full of blood is retrieved, blot the blood so collected on to the sample pad in the sample well 'A' (This delivers approximately 5µl of the whole blood specimen).

OR

In case finger prick blood is being used, touch the sample applicator to the blood on the finger prick ensuring that a loop full of blood is retrieved, immediately blot the specimen on to the sample pad in the sample well 'A' (Care should be taken that the blood sample has not clotted and the transfer to the sample pad is immediate).

OR

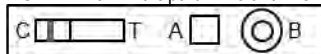
Alternatively, 5 µl of the anti coagulated or the finger prick specimen may be delivered to the sample pad in the sample well 'A' using a micro pipette.

**NOTE:** Ensure that the blood from the sample applicator has been completely taken up by the sample pad.

6. Dispense **two** drops of the clearing buffer into well 'B' by holding the plastic dropper bottle vertically.

7. At the end of **20 minutes**, read the results as follows:

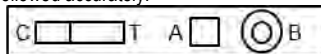
**NEGATIVE** for *P. falciparum* malaria: Only one pink-purple colored band appears in the control window 'C'.



**POSITIVE** for *P. falciparum* malaria: In addition to the control band, a distinct pink-purple colored band also appears in the test window 'T'.



**INVALID:** The test should be considered invalid if no bands appear on the device. Repeat the test with a new device ensuring that the test procedure has been followed accurately.



### PERFORMANCE CHARACTERISTICS

1. In an independent study, a panel of 167 samples whose results were earlier confirmed with expert microscopy were tested with Rapid Test for *P. falciparum* Malaria and the results obtained are as follows:

Sample type	Total no. of samples tested	Rapid Test for <i>P. falciparum</i> Malaria		Sensitivity %	Specificity %
		Positive	Negative		
<i>P. falciparum</i> positive	74	73	1	98.6	-
<i>P. vivax</i> positive	8	0	8	-	100
Malaria negative	85	1	84	-	98.8

2. In an another independent study, 125 patient samples from a *P. falciparum* endemic area were tested with Rapid Test for *P. falciparum* Malaria and microscopy (thick and thin smear). Rapid Test for *P. falciparum* Malaria was found to be 100% sensitive and 100% specific to *P. falciparum* against microscopy. All the 31 samples that tested positive for *P. falciparum* under microscopy showed positive results with Rapid Test for *P. falciparum* Malaria. The four *P. vivax* positive samples and the 90 malaria negative samples tested negative with Rapid Test for *P. falciparum* Malaria.

3. In a third independent study, 100 patient samples were tested with Rapid Test for *P. falciparum* Malaria, with another immunochromatographic test for *P. falciparum* and with microscopy. Rapid Test for *P. falciparum* Malaria showed a 99% correlation with microscopy and a 98% correlation with the other immunochromatographic test.

4. From the above results and the results of in house data, Rapid Test for *P. falciparum* Malaria is a highly sensitive and specific test for the diagnosis of *falciparum* malaria.

### LIMITATION OF THE TEST

- As with all diagnostic tests, the test result must always be correlated with clinical findings.
- The results of test are to be interpreted within the epidemiological, clinical and therapeutic context. When it seems indicated, the parasitological techniques of reference should be considered (microscopic examination of the thick smear and thin blood films).
- Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
- Interference due to the presence of heterophile antibodies in patient's sample that can lead to erroneous analyte detection in immunoassay has been reported in various studies. Rapid Test for *P. falciparum* Malaria uses HETROPHILIC BLOCKING REAGENT (HBR) to inhibit majority of this interference.
- In *P. falciparum* malaria infection, HRP-2 is not secreted in gametogony stage. Hence, in "Carriers", the HRP-2 band may be absent.
- Since the Pf. HRP-2 persists for up to 2-weeks even after successful therapy, a positive test result does not indicate a failed therapeutic response.
- In case the test needs to be used to monitor success of therapy, testing is advised only from 15 days after the completion of therapy.

### BIBLIOGRAPHY

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