

Core Malaria Pan/Pv/Pf

RAPID TEST FOR MALARIA Pan / Pv / Pf- (Device)

Ref: MAL-190026

INTRODUCTION

Core Malaria Pan/Pv/Pf is a rapid self-performing, qualitative, two site sandwich immunoassay utilizing whole blood for the detection of *P. falciparum* specific histidine rich protein-2 (Pf HRP-2), *P. vivax* specific pLDH and pan malaria specific pLDH. The test can be used for the specific detection of *P. falciparum* and *P. vivax* malaria, differentiation of other malarial species and for the follow up of antimalarial therapy.

SUMMARY

Four species of the Plasmodium parasites are responsible for malaria infections in human viz. *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Of these, *P. falciparum* and *P. vivax* are the most prevalent. Early detection and differentiation of malaria is of utmost importance due to incidence of cerebral malaria and drug resistance associated with falciparum malaria and due to the morbidity associated with the other malarial forms. As the course of treatment is dependent on the species, differentiation between *P. falciparum* and *P. vivax* is of utmost importance for better patient management and speedy recovery.

In **Core Malaria Pan/Pv/Pf** the detection system for *P. falciparum* malaria is based on the detection of *P. falciparum* specific histidine rich protein -2 (Pf HRP-2) which is a water soluble protein that is released from parasitised erythrocytes of infected individuals. The detection system of *P. vivax* is based on the presence of *P. vivax* specific pLDH. Further the detection of other malarial infections such as *P. ovale* and *P. malariae* is achieved through the pan malaria specific pLDH.

Since pLDH is a product of viable parasites, the pan band may also be used to monitor course of effective antimalarial therapy.

Core Malaria Pan/Pv/Pf detects the presence of *P. falciparum* specific Pf.HRP-2, *P. vivax* specific pLDH and pan specific pLDH in whole blood specimen and is a sensitive and specific test for the detection of all malaria species, differentiation for *P. falciparum* and *P. vivax* and monitoring successful antimalarial therapy.

PRINCIPLE

Core Malaria Pan/Pv/Pf 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 colloidal gold conjugates of anti-HRP-2 antibody, anti *P. vivax* specific pLDH antibody and anti pan specific pLDH antibody complexes the HRP-2 / corresponding pLDH in the lysed sample. This complex moves further on the membrane to the test region where it is immobilised by the monoclonal anti Pf. HRP-2 antibody and / or monoclonal anti *P. vivax* specific pLDH antibody and / or monoclonal pan specific pLDH antibody coated on the membrane leading to formation of a pink-purple colored band in the respective regions which confirms a positive test result. Absence of a colored band in the test region indicates a negative test result for the corresponding antigen. The unreacted conjugate along with the rabbit antisera colloidal gold conjugate and unbound complex if any, move further on the membrane and are subsequently immobilised 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 MATERIAL SUPPLIED

Core Malaria Pan/Pv/Pf kit contains:

A. Individual pouches, each containing:

1. Test Device: Membrane assembly pre-dispensed with monoclonal anti- HRP-2 antibody-colloidal gold conjugate, monoclonal anti *P. vivax* specific pLDH antibody-colloidal gold conjugate, monoclonal anti pan specific pLDH antibody-colloidal gold conjugate, rabbit globulin colloidal gold conjugate, monoclonal anti Pf. HRP-2 antibody, monoclonal anti *P. vivax* specific pLDH antibody, monoclonal anti pan specific pLDH antibody and anti-rabbit antibody at the respective regions.
2. Desiccant pouch.
3. 5 µl sample loop.

B. Clearing buffer in a dropper bottle.

C. Package insert.

OPTIONAL MATERIAL REQUIRED

Calibrated micro pipettes capable of delivering 5 µl sample accurately.

STORAGE AND STABILITY

The test kit may be stored between 4-30°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 in vitro diagnostic use only. NOT FOR MEDICINAL USE. Do not use beyond expiry date. Do not inter mix 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 a 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 the specimen may be stored at 2-8°C for up to 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

1. Bring the **Core Malaria Pan/Pv/Pf** kit components to room temperature before testing.
2. Open the pouch and retrieve the device, sample loop and desiccant. Check the color of the desiccant. It should be blue. If it has turned colorless or pink, discard the device and use another device. *Once opened, the device must be used immediately.*
3. Tighten the vial cap of the clearing buffer provided with the kit in the clockwise direction to pierce the dropper bottle nozzle.
4. Evenly mix the anti coagulated blood sample by gentle swirling. Dip the sample loop 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 port 'A'. (This delivers approximately 5 µl of the whole blood specimen). **OR**

In case finger prick blood is being used, touch the sample loop 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 port 'A'. (Care should be taken that the blood sample has not clotted and the transfer to the sample pad is immediate). **OR**

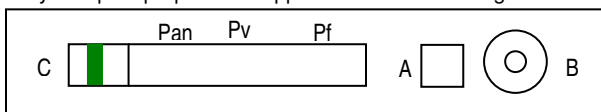
Alternatively, 5 µl of the anti coagulated or finger prick specimen may be delivered to the sample pad in the sample port 'A' using a micro pipette.

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

- Dispense four drops of the clearing buffer into port 'B', by holding the plastic dropper bottle vertically.
- At the end of 15 minutes read the results as follows:

NEGATIVE for malaria:

Only one pink-purple band appears at the control region 'C'.

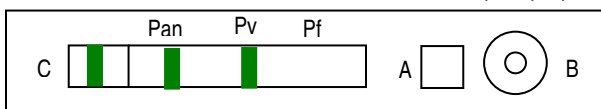


POSITIVE for malaria:

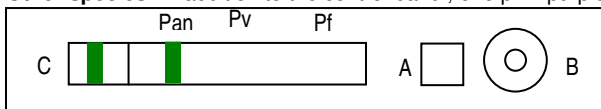
P. falciparum malaria: In addition to the control band, a pink-purple band appears at the 'Pf' and 'Pan' regions respectively.



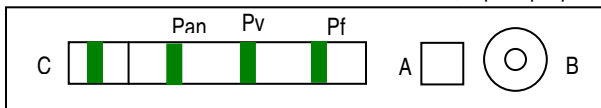
P. vivax malaria: In addition to the control band, a pink-purple band appears at 'Pv' and 'Pan' regions respectively.



Other species: In addition to the control band, one pink-purple band appears only at 'Pan' region.



Mixed infection: In addition to the control band, a pink-purple band appears at 'Pf', 'Pv' and 'Pan' regions respectively.



- 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.

LIMITATIONS 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.
- The device and buffer of different lots must not be mixed and used.
- In case of infection due to *P. vivax* or *P. falciparum*, or due to mixed infection by these species, the pan malaria band will also be positive. Hence differentiation of infection due to *P. ovale* or *P. malariae* cannot be done.
- While monitoring therapy, if the reaction of the test remains positive with the same intensity after 5-10 days, post treatment, the possibility of a resistant strain of malaria has to be considered.
- Usually, the Pv and pan bands turn negative after successful anti malarial therapy. However, since treatment duration and medication used affect the clearance of parasites, the test should be repeated after 5-10 days of start of treatment.
- In *P. falciparum* malaria infection, HRP-2 is not secreted in gametogony stage. Hence, in "Carriers", the HRP-2 band may be absent.
- HRP-2 levels, post treatment persist upto 15 days, the pan band can be used to monitor success of therapy, in *P. falciparum* malaria cases.
- In a few cases, where the HRP-2 band is positive and the pan malaria band is negative, it may indicate a case of post treatment malaria. However, such a reaction pattern may also be obtained in a few cases of untreated malaria. Retesting after 2 days is advised, in such cases.

PERFORMANCE CHARACTERISITCS

In an in house study, a panel of 251 samples whose results were earlier confirmed with microscopy were tested with Core Malaria. The results obtained are as follows:

Sample	Total No. of samples tested	Core Malaria Pan/Pv/Pf		Sensitivity	Specificity
		Positive	Negative		
P. falciparum +Ve	16	16	0	100%	-
P. vivax +Ve	25	25	0	100%	-
Malaria -Ve	210		210	-	100%

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