Core Malaria Pan/Pf

RAPID TEST FOR MALARIA

Pan / Pf

(Device)

Ref: MAL-190024

INTRODUCTION

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

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 paramount 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.

Core Malaria Pan/Pf detects the presence of pan malaria specific pLDH released from the parasitised blood cells, for the detection of all malarial parasites. Whereas, for the detection of *P. falciparum* malaria, **Core Malaria Pan/Pf** utilises 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.

In the absence of *P.falciparum* specific Pf HRP-2, the presence of the pan malaria specific band points to the presence of other malarial species; viz.; *P.vivax, P.ovale* or *P.malariae.* Speciation is done and results inferred in the context of prevalence rates of the malarial speceies prevalent in the particular region.

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

PRINCIPLE

Core Malaria Pan/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 monoclonal anti HRP-2 specific / anti pan specific colloidal gold conjugate antibodies complexes the proteins in the lysed sample. This complex moves further on the membrane to the test region where it is immobilised by the monoclonal anti HRP-2 / anti pan specific antibody coated on the membrane leading to formation of pink-purple colored band/s which confirms a positive test result. While both the bands will appear at the test region in falciparum positive samples, only one band would appear in non-falciparum malaria positive samples. Absence of this colored band/s in the test region indicates a negative test result.

The unreacted conjugate along with the rabbit globulin 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/Pf kit contains:

A. Individual pouches, each containing:

1. Test Device: Membrane assembly predispensed with monoclonal anti HRP-2 -colloidal gold conjugate, monoclonal anti pan specific pLDH -colloidal gold conjugate, rabbit globulin–colloidal gold conjugate and monoclonal anti HRP-2 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/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

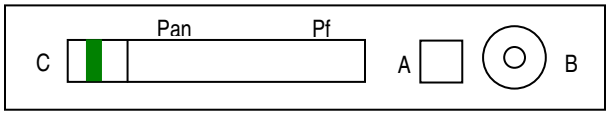
Alternatively, 5 µl of the anticoagulated or finger prick specimen may be delivered to the sample pad in the sample port 'A' using a micropipette.

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

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

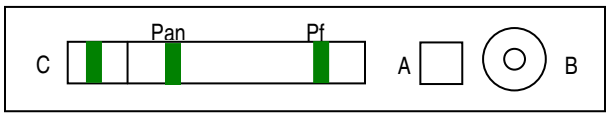
NEGATIVE for malaria:

Only one pink-purple band appears in the control window 'C'.

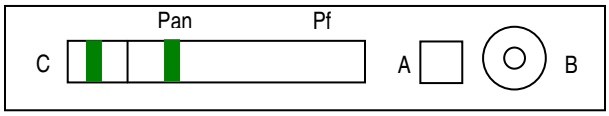


POSITIVE for malaria:

***P. falciparum* or mixed infection:** In addition to the control band, two pink-purple bands appear at regions 'Pf' and 'Pan' in the test window 'T'.



Other species (Non falciparum): In addition to the control band, one pink-purple band appears only at region 'Pan' in the test window 'T'.



6. The test result should not be interpreted after 15 minutes.
7. **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

1. As with all diagnostic tests, the test result must always be correlated with clinical findings.
2. 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).
3. Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.
4. The device and buffer of different lots must not be mixed and used.
5. In case of mixed infection (*P. falciparum* with other malarial species), both, Pf and pan malaria band will be positive. Hence differentiation of infection due to *P.vivax, P.ovale* or *P.malariae* cannot be done.
6. While monitoring therapy, using the pan band, 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.
7. Usually, the pan band 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.
8. In *P. falciparum* malaria infection, HRP-2 is not secreted in the gametogony stage. Hence, in "Carriers", the HRP-2 band may be absent.
9. 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.
10. 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 inhouse study, a panel of 251 samples whose results were earlier confirmed with microscopy were tested with **Core Malaria Pan/Pf**. The results obtained are as follows:

Sample	Total No. of samples tested	**Core Malaria Pan/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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Aspect Court, 4 Temple Row
Birmingham B2 5HG
UNITED KINGDOM