

Malaria Pan/Pf Rapid Device

A rapid, qualitative, two site sandwich immunoassay, 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 anti-malarial therapy. For professional *in vitro* diagnostic use only.

CAT. NO. PRODUCT DESCRIPTION
8/606 Malaria Pan/Pf Rapid Device – 25 Tests

INTRODUCTION

Intended Use

Four species of the Plasmodium parasites are responsible for malaria infections in humans: *P. falciparum* (*P.f.*), *P. vivax* (*P.v.*), *P. ovale* and *P. malariae*. Of these *P.f.* and *P.v.* 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.

The BIOTEC Malaria Pan/Pf Rapid Device 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.f.* malaria, the rapid device utilises the detection of *P.f.* 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.f.* specific Pf HRP-2, the presence of the pan malaria specific band points to the presence of other malarial species: *P.v.*, *P. ovale* or *P. malariae*. Speciation is done and results inferred in the context of prevalence rates of the malarial species in the particular region. Since pLDH is a product of viable parasites, the pan band may also be used to monitor success of antimalarial therapy.

Principles of the Method

The BIOTEC Malaria Pan/Pf Rapid Device is a rapid immunochromatographic test. As the test sample flows through the membrane assembly, after addition of the clearing buffer, the coloured

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 coloured 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 coloured 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.

PRODUCT CONTENTS

Each kit contains:

1. Individual pouches, each containing:
 - Test Device: Membrane assembly pre-dispensed with monoclonal anti Pf 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.
 - Desiccant pouch.
 - 5 µl sample loop.
2. Clearing buffer in a dropper bottle.
3. Instructions for use.

ITEMS REQUIRED BUT NOT PROVIDED

1. Calibrated micropipette capable of delivering 5µl sample accurately (optional).
2. Specimen collection container.
3. Timer.

STORAGE AND SHELF LIFE

1. Store as packaged in the sealed pouch at 4-30°C.
2. The test device is stable until the expiration date printed on the sealed pouch.
3. The test device must remain in the sealed pouch until use.
4. DO NOT FREEZE.
5. Do not use after the expiration date.
6. Do not re-use device.

WARNINGS & PRECAUTIONS

1. For professional *in vitro* diagnostic use only. NOT FOR MEDICINAL USE.
2. Read the instructions carefully before performing the test.
3. Do not mix reagents from different lots.
4. Handle all specimens as potentially infectious.
5. Do not eat, drink or smoke in the area where the specimens and kits are handled.
6. Do not use if pouch or devices are damaged or if any lines are visible on the device before contact with the specimen.
7. Follow standard bio-safety guidelines for handling and disposal of potentially infective material.
8. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
9. The used test should be discarded according to local regulations.
10. When used according to the instructions this product presents little risk to the user. However, take note of the following product information. Hazard: **Xn** (harmful). **R22** (harmful if swallowed). **S23** (do not breathe spray), **S46** (if swallowed seek medical advice immediately and show the label), **S61** (avoid release to the environment. Refer to safety data sheet).

SPECIMEN 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.

PROCEDURE

1. Bring the kit components to room temperature before testing.
2. Open the pouch and retrieve the device, sample loop 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.*
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 it collects on to the sample pad in the sample port 'A'. (This delivers approximately 5µl of the whole blood specimen)

OR

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

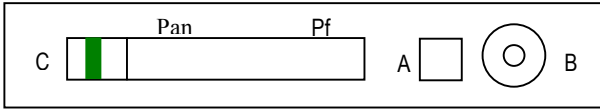
NOTE: Ensure the blood from the sample applicator pipette has been completely taken up by the sample pad.

5. Dispense four drops of the clearing buffer into port 'B', by holding the plastic dropper bottle vertically.
6. Read the results at the end of 15 minutes. However, if the background of the test window has not cleared within this time, wait for another 15 minutes before reading the results as follows:

INTERPRETATION

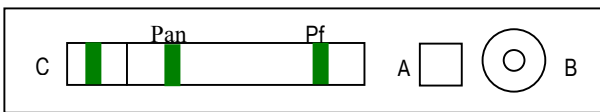
Negative for malaria:

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

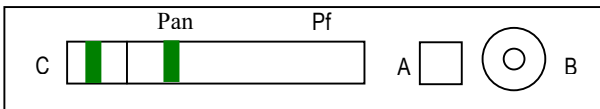


Positive for malaria:

***P. falciparum* or mixed infection:** In addition to the control band, two pink / 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 / pink-purple band appears only at region 'Pan' in the test window 'T'.



The test should be considered invalid if no bands appear or the control line is absent on the device. Repeat the test with a new device ensuring that the test procedure has been followed accurately.

Limitations

- 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, reference parasitological techniques 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 mixed infection (*P.f.* with other malarial species), both *P.f.* and pan malaria bands will be positive. Hence differentiation of

infection due to *P. vivax*, *P. ovale* or *P. malariae* cannot be done.

- 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.
- Usually, the pan band turns 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.f.* malaria infection, HRP-2 is not secreted in the gametogony stage. Hence, in "carriers", the HRP-2 band may be absent.
- HRP-2 levels, post treatment persist up to 15 days, the pan band can be used to monitor success of therapy, in *P.f.* 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. Re-testing after 2 days is advised, in such cases.
- Most blood samples clear within the running time of the test. However, in a few fresh samples and especially in stored samples, the background clearance may be delayed for 15-20 minutes longer. In such cases it is strongly recommended to extend the reading time by another 15 minutes so the results can be interpreted against a clear background.

PERFORMANCE CHARACTERISTICS

In an in-house study, a panel of 251 samples whose results were earlier confirmed with microscopy were tested with the BIOTEC Malaria Pan/Pf Rapid Device. The results obtained are as follows:

Sample	No. samples	Malaria Pan/Pf Rapid Device		Sensitivity	Specificity
		Pos	Neg		
P.f. pos.	16	16	0	100%	-
P.v. pos.	25	25	0	100%	-
Malaria negative	210	0	210	-	100%

REFERENCES

1. Howard, R.J., *et al*, 1986: Secretion of a Malarial Histidine-rich Protein (Pf. HRP II) from *Plasmodium falciparum*-infected Erythrocytes. *J. Cell Biol.*, 103, 1269-1277.
2. Rock, E.P., *et al*, 1987: Comparative Analysis of the *Plasmodium falciparum* Histidine-Rich Proteins HRP-I, HRP-II, and HRP-III in Malaria Parasites of Diverse Origin. *Parasitol.*, 95, 209-227.
3. Parra, M.E., *et al*, 1991: Identification of *Plasmodium falciparum* Histidine-Rich Protein 2 in the Plasma of Humans with Malaria. *J. Clin. Microbiol.*, 29, 1629-1634.
4. Rodriguez-Del Valle, M., *et al*, 1991: Detection of Antigens and Antibodies in the Urine of Humans with *Plasmodium falciparum* Malaria. *J. Clin. Microbiol.*, 29, 1236-1242.
5. Makler, M. T., *et al*. (1993) Parasite lactate assay as an assay for *Plasmodium falciparum* drug sensitivity. *Am. J. Trop. Med. Hyg.* 48(6), 739-741.
6. Piper, R. C., *et al.*, (1999) Immuno-capture diagnostic assays for malaria utilizing *Plasmodium* Lactate Dehydrogenase (pLDH) *Am. J. Trop. Med. Hyg.* 60(1) 109-118.
7. Srinivasan. S., *et al.*, (2000) Comparison of blood – film microscopy, The OptiMAL dipstick, Rhodamine- 123 fluorescence staining and PCR for monitoring antimalarial treatment. *Annals of Tropical Medicine and Parasitology*, 94(3) 227-232.
8. Hunte-Cooke A., *et al.*, (1999) Comparison of a Parasite Lactate Dehydrogenase-based Immuno-chromatographic Antigen Detection assay (OptiMAL®) with Microscopy for the Detection of Malaria Parasites in Human Blood Samples. *Am J. Trop Med* 60(2). 173-176.
9. John, S. M., *et al.*, (1998) Evaluation of OptiMAL, a dipstick test for the diagnosis of malaria. *Ann. Trop. Med. Parasitol.*, 92, 621-622.
10. Quintana M., *et al.*, (1998) Malaria diagnosis by dipstick assay in a Honduran Population with coendemic *Plasmodium falciparum* and *Plasmodium vivax*. *Am. J. Trop. Med. Hyg.* 59(6) 868-871.
11. Palmer, C. J., (1998) Evaluation of OptiMal test for rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum*. *J. Clin Microbiol.* 36(1) 203-206.
12. Moody A., *et. al* (2000) Performance of the OptiMAL® malaria antigen capture dipstick for malaria diagnosis and treatment monitoring. *British Journal of Hematology*, 109, 1-5.

KEY TO SYMBOLS



Temperature limitation



Use by/Expiry date

IVD

In Vitro Diagnostic



Harmful (Xn)